

A minimalistic approach to develop new anti-apicomplexa polyamines analogs.

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Abstract:

The development of new chemical entities against the major diseases caused by parasites is highly desired. A library of thirty diamines analogs following a minimalist approach and supported by chemoinformatics tools have been prepared and evaluated against apicomplexan parasites. Different member of the series of N,N'-disubstituted aliphatic diamines shown *in vitro* activities at submicromolar concentrations and high levels of selectivity against *Toxoplasma gondii* and in chloroquine-sensitive and resistant-strains of *Plasmodium falciparum*. In order to demonstrate the importance of the secondary amines, ten N,N,N',N'-tetrasubstituted aliphatic diamines derivatives were synthesized being considerably less active than their disubstituted counterpart. Theoretical studies were performed to establish the electronic factors that govern the activity of the compounds.

Keywords: N,N'-disubstituted Diamines, Polyamines, Anti-apicomplexa, NTDs, Cheminformatics.

1. Introduction

The phylum *Apicomplexa* includes intracellular protozoan pathogens responsible for many diseases with huge global significance in the medical and veterinary field. *Plasmodium spp* is an apicomplexa parasitic alveolate and is the cause of malaria in different parts of the world. Malaria afflicts millions of individuals in the tropical zones, causing serious morbidity and mortality. *Toxoplasma gondii* is the etiological agent of toxoplasmosis. This opportunistic pathogen has worldwide distribution, may cause cerebral pathology in immunocompromised individuals, and can produce several congenital anomalies including induction of miscarriage and neurological disorders.^{1,2} *T. gondii* is one of the leading causes of focal central nervous system (CNS) disease in AIDS patients. Other apicomplexan organisms such as *Cryptosporidium parvum* and *Cyclospora spp.* also cause opportunistic infections in humans and have relevance in developing countries by causing diarrhoea outbreaks.^{3,4}

Other members of this phylum including *Theileria*, *Sarcocystis* and *Babesia spp.* are important pathogens of animals. The limited arsenal of drugs available against these organisms is insufficient due to the rapid emergence of resistance, as is the case for *Plasmodium* or incomplete activity, as the case for chronic *Toxoplasmosis*. Thus, there is a continuous need develop new chemotherapies in order to combat these diseases in a unique mode of action.^{5,6}

Significant efforts have been undertaken in the last decades to eradicate malaria.⁷ Between 2000 and 2015 there was a registered reduction in malaria total estimated cases of more than 14% in 43 out of the 99 countries with transmission. Due to the development of resistance, antimalarial drugs which have been used to date since the advent of chloroquine, have lost effectiveness around the World.⁸ Currently, artemisinin-based combination therapy (ACT) is the mainstay of malaria treatment. Unfortunately, there are an increasing number of countries (mostly in East Asia) in which resistance to artemisinin has been reported.^{9,10} Additionally, there is an alarming emergence of multiresistant strains in distinct parts of the globe. Furthermore, the resistance in the mosquito vector to pyrethroids has also been reported.¹¹ This scenario has led governmental and non-governmental organizations to focus their efforts to finding new compounds that serve to combat the disease.¹²

Polyamines are essential metabolites that are implicated in many physiological functions including DNA replication and repair, being essential for normal cell growth and viability.¹⁴ The apparent universal distribution of polyamines, and the complexity of compensatory mechanisms that are involved to maintain polyamine homeostasis, suggest that these molecules are critical for cell survival.

The biosynthesis of polyamines typically starts with the conversion of arginine to putrescine which occurs through one of two distinct pathways: arginase coupled with ornithine decarboxylase (ODC), or arginine decarboxylase (ADC) coupled with agmatine iminohydrolase. Putrescine can be further converted into spermidine and spermine by spermidine and spermine synthases, respectively. When polyamines are available, spermine or spermidine can be retro-converted into spermidine and putrescine by the consecutive action of spermine/spermidine N¹-acetyltransferase (SSAT) and polyamine oxidase (PAO).¹⁵⁻¹⁷

The phylum *Apicomplexa* has a polyamine metabolism that involves a combination of several sensitive feedback systems regulating their synthesis, degradation, and transport.^{18,19} Regulation of polyamine biosynthesis is complex and the key biosynthetic enzyme, ODC is one of the most highly regulated enzymes. It is present in *P. falciparum*, but extracts of *T. gondii* had no detectable ODC and ADC activity. Thus, *T. gondii* lacks a forward-directed polyamine biosynthetic pathway and elucidate the need to discover an alternate mechanism to tackle the pathogenicity of this organism.¹⁹

Previously reported studies from Bitonti *et al* shown a series that bis-(benzyl)-polyamines analogs with antimalarial activity against both, a chloroquine-sensitive and -resistant strains of *P. falciparum*.²⁰ A selected analog of the series, MDL-27695, (**Figure 1**) was markedly active and presented a synergistic effect with D,L- α -difluoromethylornithine (DFMO), being able to cure *P. berghei* infected mice. Initially, MDL-27695 was proposed to target the polyamines biosynthesis. Nevertheless, the mechanism of action of these compounds looks likely to be more complex. On one hand, Bitonti *et al* observed that DNA/RNA synthesis was depleted faster with MDL-27695 than upon DFMO treatment, on the other polyamines biosynthesis was possibly repressed but might not be the only cause of the cytotoxic event.²¹

[FIGURE 1]

In previous work, we have synthesized N,N'-disubstituted aliphatic diamines and determined their activity against parasites of the kinestoplastida group.²² Many analogs were active against *T. cruzi*, *T. brucei* and *L. donovani* showing low cytotoxicity. Here, we wanted to expand the analysis of the initial collection to determine activity against *Toxoplasma* and *Plasmodium*, looking to link the activity with the pathophysiological differences between these two parasites. Moreover, we intended to understand

their mechanism of action linking their phenotypical differences with their metabolic differences. Finally, we were eager to determine the relationship between the physicochemical properties and activity of the relevant compounds.

2. Results and discussion

2.1. Chemistry

The molecular scaffold of our library was based on the assumption that MDL-27695 interacts with a molecular target anchored by two nitrogens requiring a bulky hydrophobic substituent in both terminal nitrogens (**Figure 1**). Following that hypothesis, a minimal version of that scaffold should be a bis-substituted benzyl N,N'-disubstituted diamine. That analog should fulfil the steric and polar requirements and at the same time be considerably simpler. The library has two sources of diversity: 1) the carbon size chain, which brings flexibility and more grades of freedom and 2) the substituent on the aromatic ring. The library was prepared in a single step by a double reductive amination between the diamines and the benzaldehydes using the procedure by Abdel-Magid *et al*²³ with minimal modifications (**Scheme 1**). The library was built with all the possible combinations of commercial aliphatic diamines with a 3, 4, 6, 8, 10 and 12 carbon chain length and five aromatic aldehydes (benzaldehyde, 4-methoxy-benzaldehyde, 4-methoxy benzaldehyde, 3-hydroxy-4-benzyloxy benzaldehyde and 3-methoxy-4-benzyloxy benzaldehyde). The library of thirty N,N' disubstituted diamines was prepared in solution, including the dodecyldiamine analogs that were not previously reported.²²

The synthesis produced all the desired products with good yield (**Table 1**, average 77%, ranging 30 to 99%). The yield was similar across each series (around 80 %).

[SCHEME 1]

2.2. Biological evaluation

The polyamines pathway in *Plasmodium spp.* has been studied during the last 25 years with different grades of success. Target-oriented drug design directed to enzymes in the polyamine pathway provided promising hits, without showing considerable progress towards becoming lead compounds.

Therefore, having in mind all the successful examples via phenotypic screening, and looking to a wide spectrum candidate, we decided to assay our collection not only against *P. falciparum* strains but also against *T. gondii*. Also, cytotoxicity was evaluated in two different cell lines, African green monkey kidney epithelial (VERO) cells and human fibroblasts.

2.2.1. Antiplasmodium activity

All the analogs were assayed against the chloroquine sensitive Sierra Leona clone (D6) and the chloroquine resistant, Indochina clone (W2) strains of *P. falciparum*. To our satisfaction, most of the compounds were active against both the strains. (**Table 1**). Ninety percent of the collection has an IC_{50} below 5 μ M, with 73% of the library being more potent than MDL-27695. In general, there are no substantial variations in the potency between the sensitive and resistant strain, suggesting that the mechanism that confer chloroquine resistance to the W2 strain does not affect sensitivity to this family of compounds.

[TABLE 1]

When comparing activities based on the benzyl substituent some clear tendencies emerge. First, there is a narrow difference between the more and the less potent member of each series, which ranges from 7.61 for R=4-BnO to 1.02 for R=3-MeO,4-BnO (**Figure 2**).

[FIGURE 2]

These slight differences in antiplasmodium efficacy of the analogs indicate that the diamine chain length does not have a strong effect the activity. At the same time, comparing the activity by the same diamine linker it seems that it does not considerably affect the potency.

The analysis of the unsubstituted benzyl derivatives (**1** to **6**) reveal that compounds with large chains (compounds **4**, **5** and **6**) are very potent (below 1 μ M against both strains). A comparison with our template compound MDL-27695 reveal that these analogs are 3 to 8 times more active being shorter in length and having only two nitrogen atoms. The R=4-MeO series exhibited a similar activity profile,

but in this case, compound **11** with a C10 diamine was the most active with IC_{50} of 0.60 μ M. A different activity profile was noticed in case of 4-benzyloxy analogs (**13** to **18**). The tendency changes, with **15** was the most active ($n=8$, $IC_{50}=0.39$ for D6 and 0.29 μ M for W2) and **18** was the least active ($n=12$, $IC_{50}=8.00$ μ M for both D6 and W2 strains).

Isovanillin analogues **19**, **20** and **21** did not show activity up to the maximum concentration tested (8.00 μ M). That anomaly could be related to the presence of a 3-OH group, which by substantially increasing hydrophilicity would alter membrane permeability. Compound **27** was the most active analog of the whole library with an IC_{50} of 260 nM against the *P. falciparum* D6 strain and SI >31.7, only 3-fold less active than chloroquine and artemisinin and 12-fold more active than MDL-27695. However, compound **4** showed an IC_{50} of 370 nM but presents an even higher SI (>39.7).

As for the resistant strain analogue **5**, which is 1.5-fold more active than chloroquine, combines the best activity and selectivity, with an IC_{50} 280 nM. Finally, the 3-methoxy-4-benzyloxy benzyl analogs are the most active series, with an average of 0.62 μ M, with compound **27** ($n=6$, $IC_{50}=0.26$ μ M) the most active and **30** the less active ($n=12$, $IC_{50}=1.28$ μ M). Interestingly, there is slight difference activity among the compounds in this series, suggesting that increasing the chain has minimal effect on activity.

Having completed the analysis of the chain length and the aromatic ring substitution influence on the antiplasmodial activity, it was important to establish the impact of the nitrogen substitution. There are many literature reports of monosubstituted diamines, but surprisingly, no precedents of bioactive tetrasubstituted aliphatic diamine analogs as antiparasitic agents.²⁴ With that idea in mind, ten tetrasubstituted analogs were prepared. Only the simplest aromatic substituents ($R=H$ and 4-OMe) and five diamines ($n=3,4,6,8$ and 10) were selected, looking to comply with Lipinsky's rules ($MW<500$ Da and $\log P<5$), additionally dodecylamine analogs were not considered because they were, on average, less active. In this opportunity, a different methodology for the synthesis was required. A one-pot procedure was selected, where two consecutive reductive aminations occurred using sodium triacetoxy borohydride, acetic acid in DCM at room temperature.^{25,26} Ten new products were prepared (Scheme 2) with 22% global yield (Table 1). The main drawback of the reaction was the aldehyde reduction that forced using at least 4.5 equivalents of that component. Unfortunately, when more equivalents were used, the amount of the benzyl alcohol increased, impeding a clean separation of the product by column chromatography.

[SCHEME 2]

The new analogs were evaluated against the D6 and W2 strains of *P. falciparum* and did not show activity at the maximum concentration tested (8 μ M). A more detailed analysis revealed that the most active member of each series of the disubstituted analogs were at least 40 times (Compound **4**, R=H, IC₅₀ *P.f.* D6 = 0.37 μ M) and 19 times (compound **11**, R=4-MeO, IC₅₀ *P.f.* D6 = 0.60 μ M) more active than the tetrasubstituted analogs **34** and **40** (both *P.f.* D6 > 8.0 μ M), respectively.

The antimalarial activity of N,N-disubstituted putrescine and trimethylenediamine had been previously reported.²⁷ Comparing the activity of the reported N,N-disubstituted with the corresponding N,N'-disubstituted analogs has shown that the last ones were always more active (between 1.5 to 7 times more potent). These results clearly demonstrate that the secondary amine is an important structural feature required for the antimalarial activity, being the N,N'-disubstitution the pattern that provided the best results.

2.2.2. Anti-Toxoplasma gondii activity.

To test activity of our library of diamines against *T. gondii* we cultured parasites on human foreskin fibroblasts in the presence of drug at 1 μ M or of DMSO as solvent control for 5 days, at which time point cells were fixed and stained to monitor parasite growth. The results, presented in **Table 1**, show that in general the molecules in our library are not as effective in inhibiting *T. gondii* growth as they are with *P. falciparum*. Compounds **5**, **12**, **13**, **17** and **25** (20 % of the collection) were the most effective against *T. gondii* and exhibited at least 75% of inhibition compared with the untreated parasites. Interestingly, compound **5** was also the most active member of the benzyl series against *P. falciparum* (IC₅₀=0.48 μ M). Compound **12** is the most active on *T. gondii* of the 4-methoxy benzyl series, and is just two carbons larger than the analog of that family with best antiplasmodial activity. The 4-benzyloxy benzyl containing compounds **13** and **17**, which have 3 and 10 carbon chain respectively, show 75 % of *T. gondii* growth inhibition. Compound **15**, which did not affect *T. gondii* growth was the member of that series (4-benzyloxy benzyl) with the best antiplasmodial. The 3-methoxy-4hydroxy benzyl and the 3-methoxy 4-benzyloxy benzyl series contains a second oxygen on

the benzyl ring. For the isovanillin analogues, short chains derivatives (**20**, **21**) were the most active, behaving differently on *P. falciparum* were the longer analogs were the most potent.

Finally, the IC₅₀ of most active analogs was determined, revealing that compounds **13** and **25** has an IC₅₀= 2.97 μ M and **25**, the most active member of the series has an IC₅₀= 1.53 μ M. Interestingly, none of these compounds displayed cytotoxicity on fibroblast at 10 μ M, providing an SI as anti-*T. gondii* of 3.37 and 6.54 for **13** and **25**, respectively. In summary, the overall library behavior was different between the two parasite species, showing that tachyzoites were not affected in the same way that *Plasmodium* erythrocytic stage (trophozoites) suggesting a different action mechanism in both parasites.

2.2.3. Cytotoxicity.

Vero cells are an excellent model for test cytotoxicity *in vitro* because is an aneuploid and a continuous cell lineage. The library was tested for their effect on Vero cells to a maximum concentration of 8 μ M. Only five compounds, (**9**, **13**, **14**, **25** and **28**), which corresponds to 16 % of our library, were cytotoxic with a range of IC₅₀ between 4 to 8 μ M. These five diamines analogues have a short carbon chain length, and all, with the exemption of compound **9**, have benzyloxy substituents on position 4 of the aromatic ring. Nonetheless, these five compounds, particularly **25** and **28** exhibit excellent activities against apicomplexa parasites. Therefore, even being cytotoxic at low micromolar they are 10-fold higher as antimalarial providing good SI (15.9 and 14.6, respectively).

2.3. Cheminformatics and clusters analysis.

The rationale used to design this targeted library of symmetrical N,N'-disubstituted diamines allowed only two entries for scaffold modifications providing a collection that has low chemical diversity. However, using the ChemMineTools online platform,²⁸ it was possible to cluster the collection based on their structural matrixes. This information was useful to establish a structural-activity relationship correlating the activity and topological parameters. (**Figure 3**). The outcome of this calculation revealed the precedence of three main clusters (marked in green, red and blue in **Figure 3**) and ten secondary clusters (labelled from A to J in **Table 1**).

The main cluster (green) contains the most active analogs in both parasites. This cluster has two sets of subclusters: one is represented for compounds **13**, **14**, **25** and **26** (clusters G and H) and the second is represented by **15**, **16**, **17**, **18**, **27**, **28**, **29** and **30** (clusters I and J). When activities are considered,

the first subcluster includes analogs with the highest potency towards both parasites. The second subcluster is more active against *P. falciparum*. The percentage of growth inhibition of *Toxoplasma* is low with the exception of compound **17**.

[FIGURE 3]

2.3.1. Physicochemical characteristics and ADME-Tox.

Addressing pharmacokinetic properties at the early stages of drug development reduces the chances of failure on clinical trials. To characterize the profile of our analogs, we performed computational studies of all compounds to predict their adsorption, distribution, metabolism and excretion (ADME) properties, Lipinski's rule of five, toxicity liabilities and drug likeness. The *in silico* toxicology calculated by the OSIRIS property explorer platform²⁹ indicates that none of the products prepared are potentially mutagenic, irritant, teratogenic, or toxic for sexual reproduction. The only exception is the isovanillin subfamily derivatives, which are potentially mutagenic. Likewise, this subset demonstrated to possess the lowest levels of biological activities, therefore they are not good candidates for future optimizing steps.

On the other hand, the most relevant parameters (molecular weight, polar surface area, volume, logBB and logP) of the collection allow us to predict the potential of the compounds as leaders, possible administration routes and to enrich SAR. To that purpose the Molinspiration³⁰ and ChemAxon³¹ platforms were used to calculate these physicochemical parameters including the predominant molecular form at physiological pH (**Table 2**).

According to Lipinski's rule of five, a potential drug candidate should be orally active if the molecular weight is ≤ 500 Da, $\log P \leq 5$, the number of hydrogen bond acceptors ≤ 10 and the number of hydrogen bond donors ≤ 5 . The analysis of the collection revealed that 66.6 % of the collection has $MW < 500$ (256 to 654 Da) and 83.3 % has $\log P < 5$ (-1.14 to 7.79). (**Figure 4**)

According to Hammett and Hansch's principle, a relationship between lipophilicity descriptors like logP and biological activity most commonly produce a parabolic correlation. In the case of our collection, the logP values of the 30 compounds fluctuate between -1.14 and 7.79. A correlation is clearly observed for *P. falciparum* (**Figure 4**), where most active analogs have logP between 2.47 and

4.95. By contrast, the correlation for *T. gondii* is not clear (**Figure 5**). However, we could constrain the logP range values between 2.66 to 3.75 for the highest values of activity against *T. gondii*.

However, our library contains ionizable groups that are charged at physiological pH, which make logD a better descriptor of the lipophilicity of these molecules. The calculated logD at physiological pH (7.4) of the collection is presented in **Table 2**, with 90 % of the collection being ≤ 5 (-1.09 to 5.92). Given the chemical nature of the amine groups in the designed structures, the lipophilicity decreases markedly as the pH drops. This fact would suggest that the molecule could have a better interaction in the acidic environment of some organelles, in the same way as ethambutol does within lysosomes and autophagosomes.³² In summary, this analysis reveals that most of the compounds has good potential to be orally active drug candidates.

The distribution of the compounds between blood and brain is a very important consideration for new candidate drug molecules. In case *T. gondii*, which effectively infects neurons and causes disease in the brain, good drug candidates must be able to cross the blood brain barrier (BBB). To predict that properly we calculated the logBB using COSMOquick.³³ This calculation shows that 75% of our compounds have logBB > 0.3. It has been shown that molecules with logBB > 0.3 cross the BBB readily while molecules with logBB < -1 are poorly distributed to the brain,^{34,35} thus our library includes numerous compounds that would be expected to enter the brain.

[FIGURE 4]

[FIGURE 5]

[TABLE 2]

2.4. Analysis of the molecular electrostatic potential of the aromatic portion.

Molecular electrostatic potentials (MEPs) were used for analyzing drug-receptor and enzyme-substrate interaction and other recognition processes, based on the assumption that those potentials described how different molecular regions will interact with other approaching chemical species.

In order to determine how the electronic potential of the aromatic portion of the analogs affects the activity, a tridimensional molecular electrostatic potential map of the aromatic portion of the prepared N,N'-dibenzyl aliphatic diamines derivatives were performed at the van der Waals contact surface. To simplify the calculations, only substituted N-methyl benzyl were used (**Figure 6a**. R=H; R=4-OMe; R=4-OBn; R=3-OH,4-OMe; R=3-MeO,4-BnO). The geometries of the groups were optimized using B3LYP functional along with 6-311++G(d,p). (**Figure 6b**) The electrostatic potentials, superimposed onto a surface of constant electron charge density were calculated (0.001 e/au^3 , **Figure 6c**).

It is well known that electrostatic potential has been defined as the energy of interaction of a positively charge point with the nuclei and the electrons of a molecule. On **Figure 6C** the coloring area represents the electrostatic potential, $V(r)$, providing a measurement of the molecular charge distribution. The calculated MEP maps for the five analogs that go from -0.1408 au in deepest red for the strongest attraction, to $+0.1152 \text{ au}$ in deepest blue for the strongest repulsion. Negative regions $V(r)$ are usually associated with a lone pair of electronegative atoms and the π electrons of the unsaturated hydrocarbons. In this case, the MEPs are different for each of the five analogs studied and, as expected, is affected by the aromatic ring substituents. The negative potentials are localized at the oxygens of the phenols and methoxy groups. On the other side, the positive potential is localized on the nitrogen amine and is significantly affected by the substituent on the aromatic ring.

When the activity of each series was correlated with the electrostatic potential, two clear tendencies emerged. On one side, the activity of the analogs with benzyloxy group seems governed by steric effects. On the other side, the activity of the series is clearly associated to the negative electrostatic potential, being detrimental to the antiplasmodial activity.

[FIGURE 6]

3. Conclusions

Enzymes are usually arranged into symmetrical homodimers or tetramers, with the consequent active site organization in a highly symmetrical fashion. Thus, symmetrical inhibitors or biological actuators will correspond generally to their binding site.³⁶ Numerous examples of symmetrical active compounds have been reported including anticancer,^{37,38} antiparasitic,^{39,40} antitubercular⁴¹ and enzyme

inhibitors,^{42,43} between others. The compounds designed and prepared on this work were inspired on the symmetrical compound MDL-27695. We were looking to simplify that compound's structure, removing nitrogens from the carbon chain and inquiring the effect of oxygenated substituents of the aromatic ring on the activity. Therefore, we searched for a fast and easy way to explore the chemical space of the proposed disubstituted N,N'-aliphatic diamines. A major advantage of the proposed scaffold lies in the fact that the synthesis of symmetrical derivatives is extremely straightforward and only required commercially available starting materials. The synthesis did not need additional protection/deprotection steps, minimizing the synthesis to a single-step, being a clear example of atom economy and green chemistry.^{44,45}

It has been suggested that symmetry and planarity disruption is a valuable strategy to improve aqueous solubility.⁴⁶ The synthesized compounds are very flexible preventing a good packing that facilitates the dissolution process. Also, the two nitrogen atoms on the aliphatic chain provide the required polarity to improve the water solubility and bioavailability. Therefore, from the solubility point of view, the symmetrical scaffold prepared did not represent a disadvantage.

The one-pot synthetic procedure provided 30 symmetric derivatives in good yields. *In vitro* studies have demonstrated that most of the analogs (26 of 30 compounds) are active against *P. falciparum* at micromolar level or below (9 compounds at submicromolar level). On the other hand, the related parasite *T. gondii* does not seem as sensitive to these type of compounds (6 of 30 compounds shows at least 75% of activity at 1 μ M). The differences on the sensitivity between *T. gondii* and *P. falciparum* could be attributed to a favoured incorporation to the red bloods cells or the interaction with different molecular targets due to their metabolic divergences.

A series of tetrasubstituted N,N'-aliphatic diamines were prepared being considerable less active against both *P. falciparum* strains than the bis-substituted counterpart demonstrating the importance of the secondary amine on the activity.

A summary of the structure-activity analysis is detailed on **Figure 7**. As can be seen, the antimalarial activity of the compounds is fairly responsive to the structural variations in most of the series, with certain substituents increasing activity, like R=H, or R=3-MeO,4-BnO. MEPs results clearly shown that the aromatic substituents affect the electrostatic potential of the nitrogen and the aromatic ring and that, together with the steric factors, can be correlated with the activity. Chain length

appears to slightly influence potency, being the electronic and the steric the most critical factors modulating the activity.

The selection of the best drug candidate as antimalarial and anti-*T. gondii* should combine a low IC₅₀ values with Lipinski's rules compliance, low cytotoxicity (high SI) and ideal range of logBB (only for *T. gondii*). Following that premise, analogs **4**, **5**, **15**, **26** and **27** are the most promising antimalarial candidates, having submicromolar IC₅₀ and SI>20. Filtering out the selection by the ADME parameters points to compound **4** as the best candidate. In the case of *T. gondii*, analogs **13** and **25** based on the activity are the most promising, but of these only **13** is predicted to cross the BBB, which would make it the best candidate for future studies (**Figure 5**). It is likely that the mechanism of action of these compounds differs between these two parasites. Additionally, the targets and mechanisms of action in each parasite might be different across the different series. Future studies with the best candidates will focus on elucidating mode of action. The fact that similar compounds act by different mechanisms in trypanosomatids^{22,47} and the pathogenic fungus *Pneumocystis carinii*⁴⁸ support this hypothesis. Polyamine oxidase have been studied as a possible target of MDL-27695 on *P. falciparum*.⁴⁹ The oxidation of that compound by purified rat liver oxidase release benzaldehyde and the N-monosubstituted analog, then goes to another oxidation cycle releasing the free polyamine. Also, polyamine concentration increases inside *P. falciparum* infected red blood cells by newly recruited transporters.

Similar degradation products have been studied on *Plasmodium falciparum*,⁵⁰ displaying similar potency to the bis-benzyl disubstituted analogs but holding a bulky anthracene ring. Having in mind that monosubstituted diamines have been described as polyamine transport inhibitors,⁵¹⁻⁵³ a possible action mechanism could involve oxidation to activate the compound that then inhibit the polyamine transport.

In conclusion, the structural simplification of MDL-27695 provided potent antimalarial analogs validating our initial hypothesis. The fact that compounds **4** and **5**, which has strong similarities to MDL-27695 but only have 2 nitrogens, supports the idea that the bis-N-benzyl moiety could be acting as the pharmacophore within those structures. Lastly, the N,N'-disubstituted aliphatic diamine analogs provides unique tools to develop new antiparasitic agents and to study biological processes related to polyamines metabolism. A possible multitarget action mechanism should be validated by non-directed metabolomics and proteomics study.

[FIGURE 7]

4. Material and Methods

4.1. General Information

^1H and ^{13}C NMR spectra were acquired on a Bruker Avance II 300 MHz (75.13 MHz) using CDCl_3 as solvent. Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane (TMS) at 0 ppm as internal standard and coupling constants (J) are in hertz (Hz). Chemical shifts for carbon nuclear magnetic resonance (^{13}C NMR) spectra are reported in parts per million relative to the center line of the CDCl_3 triplet at 76.9 ppm. The following abbreviations are used to indicate NMR signal multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = pentet, br = broad signal. High-resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF II with lock spray source. IR spectra were obtained using an FTIR Shimadzu spectrometer and only partial spectral data are listed. Chemical reagents were purchased from commercial suppliers and used without further purification, unless otherwise noted. Solvents were analytical grade or were purified by standard procedures prior to use. Yields were calculated for material judged homogeneous by thin layer chromatography (TLC) and nuclear magnetic resonance (^1H -NMR). All reactions were monitored by thin layer chromatography performed on silica gel 60 F₂₅₄ pre-coated aluminium sheets, visualized by a 254 nm UV lamp, and stained with an ethanolic solution of 4-anisaldehyde. Column flash chromatography was performed using silica gel 60 (230–400 mesh).

4.2. *In vitro* activity against *P. falciparum* (IC₅₀).

Antimalarial activity was determined *in vitro* on the chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *P. falciparum*. The 96-well microplate assay was based on the evaluation of the effect of compounds on the growth of asynchronous cultures of *P. falciparum*, determined by the assay of parasite lactate dehydrogenase (pLDH) activity. The appropriate dilutions of the compounds were prepared in DMSO or RPMI-1640 medium and added to the cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) setup in clear flat bottomed 96-well plates. The plates were placed into the humidified chamber and flushed with a gas mixture of 90% N₂, 5% CO₂ and 5% O₂. The cultures were incubated at 37 °C for 48 h. Growth of the parasite in each well was determined by pLDH assay using Malstat reagent. The medium and red blood cells (RBC) controls were also setup

in each plate. The standard antimalarial agent chloroquine and artemisinin were used as the positive controls.

4.3 *In vitro* activity against *T. gondii*.

Toxoplasma parasites (RH strain parasites expressing the green fluorescent protein, GFP) were maintained in human foreskin fibroblast (HFF) monolayers in Dulbecco's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 g/ml streptomycin. Uninfected and infected HFFs cells were maintained in a humidified incubator at 37 °C with 5% CO₂. For primary screen, 10³ parasites were allowed to infect a confluent HFF monolayer. At 8 hours post-infection, the medium was replaced with medium containing 1 or 10 µM of each compound. Three days later cultures were monitored by fluorescence microscopy to monitor parasite growth. To determine IC₅₀ HFFs were infected with 80 parasites and after four hours extracellular parasites were washed off and media with drug at a range of concentrations was added. At 5 days post-infection, cultures were fixed with methanol for 2 minutes and stained with crystal violet to visualize plaques which were enumerated. Percentage survival was calculated by dividing the number of plaques in drug over that in equivalent amount of DMSO. Experiments were performed in three experimental and biological replicates.

4.4. Cytotoxicity assay on Vero cells.

The *in vitro* cytotoxicity was determined against mammalian kidney fibroblasts (VERO). The assay was performed in 96-well tissue culture-treated plates as described earlier. Briefly, cells were seeded to the wells of the plate (25,000 cells/well) and incubated for 24 h. Samples were added and plates were again incubated for 48 h. The number of viable cells was counted and the plot for EC₅₀ performed.

4.5. ADME/toxicity predictions.

Computational modeling to estimate the bioavailability, aqueous solubility, blood brain barrier potential, human intestinal absorption, mutagenicity, and toxicity for the compounds was performed using the ChemMineTools and Osiris Property.

4.6. General procedure for N,N-diamines disubstituted synthesis

Aldehyde (3 eq dissolved in the same mixture of solvents) was added to a solution of diamine (1 eq) in an anhydrous mixture of DCM:methanol (3:1). The mixture was refluxed overnight, the solvent was evaporated and the crude di-imine was dissolved in an anhydrous mixture of DCM:methanol (1:1).

The solution was cooled at 0 °C, and sodium borohydride (3 eq) was added portion wise. The reaction was allowed to warm to room temperature and stirred for additional 12 hours. Excess of sodium borohydride was quenched acidifying with 10% HCl, with subsequent addition of ammonium hydroxide to reach a basic pH. Phases were separated and the aqueous phase extracted with DCM (3 x 20 mL). Combined organic extracts were dried with sodium sulphate and evaporated. Products were purified by flash chromatography.

4.6.1 Synthesis of N1,N3-dibenzylpropane-1,3-diamine (1).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 97 mg of white solid (isolated yield: 79 %). ¹H NMR (CDCl₃) δ 7.31 (m, 10H, aromatics); 3.78 (s, 4H, Ar-CH₂); 2.72 (t, 4H, *J* = 6.6 Hz, N-CH₂); 1.74 (m, 2H, C2-H). ¹³C NMR (CDCl₃) δ 140.3 (C); 128.4 (CH); 128.2 (CH); 126.9 (CH); 54.1 (CH₂); 48.0 (CH₂); 30.0 (CH₂). IR ν 3296 (R-NH-R1); 3026 (Ar-H); 1119 (C-N-C); 697 (monosubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₁₇H₂₃N₂ (M+H⁺) 255.1861; found 255.1853.

4.6.2. Synthesis of N1,N4-dibenzylbutane-1,4-diamine (2).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 88 mg of white solid (isolated yield: 73 %). ¹H NMR (CDCl₃) δ 7.28 (m, 10H, aromatics); 3.78 (s, 4H, Ar-CH₂); 2.64 (t, 4H, *J*=6.6 Hz, N-CH₂); 1.57 (m, 4H, C2-H and C3-H). ¹³C NMR (CDCl₃) δ 139.6 (C); 128.4 (CH); 128.2 (CH); 127.1 (CH); 53.6 (CH₂); 48.9 (CH₂); 27.6 (CH₂). IR ν 3311 (R-NH-R1); 1120 (C-N-C); 697 (monosubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₁₈H₂₅N₂ (M+H⁺) 269.2018; found 269.2011.

4.6.3. Synthesis of N1,N6-dibenzylhexane-1,6-diamine (3).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 47 mg of white solid (isolated yield: 46 %). ¹H NMR (CDCl₃) δ 7.28 (m, 10H, aromatics); 3.78 (s, 4H, Ar-CH₂); 2.61 (t, 4H, *J*= 7.2 Hz, N-CH₂); 1.49 (m, 4H, C2-H and C5-H); 1.34 (m, 4H, C3-H and C4-H). ¹³C NMR (CDCl₃) δ 140.3 (C); 128.4 (CH); 128.2 (CH); 126.9 (CH); 54.0 (CH₂); 49.3 (CH₂); 29.9 (CH₂); 27.3 (CH₂). IR ν 3312 (R-NH-R1); 3026 (Ar-H); 1119 (C-N-C); 697 (monosubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₂₀H₂₉N₂ (M+H⁺) 297.2331; found 297.2328.

4.6.4. Synthesis of N1,N8-dibenzyl-octane-1,8-diamine (4).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 85 mg of white solid (isolated yield: 94 %). ^1H NMR (CDCl_3) δ 7.30 (m, 10H, aromatics); 3.82 (s, 4H, Ar- CH_2); 2.67 (t, 4H, $J = 7.2$ Hz, NCH_2); 1.52 (m, 4H, C2-H and C7-H); 1.30 (s, 8H, C3-H to C6-H). ^{13}C NMR (CDCl_3) δ 140.2 (C); 128.4 (CH); 128.2 (CH); 126.9 (CH); 53.9 (CH_2); 49.3 (CH_2); 29.9 (CH_2); 29.5 (CH_2); 27.5 (CH_2). IR ν 3307 (R-NH-R1); 3026 (Ar-H); 1121 (C-N-C); 697 (monosubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{22}\text{H}_{33}\text{N}_2$ ($\text{M}+\text{H}^+$) 325.2644; found 325.2660.

4.6.5. Synthesis of N1,N10-dibenzyldecane-1,10-diamine (5).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 73 mg of white solid (isolated yield: 89%). ^1H NMR (CDCl_3) δ 7.31 (m, 10H, Ar-H); 3.84 (s, 4H, Ar- CH_2); 2.65 (t, 4H, $J = 7.20$ Hz, N- CH_2); 1.55 (m, 4H, C2-H and C9-H); 1.25 (m, 12H, C3-H to C8-H). ^{13}C NMR (CDCl_3) δ 136.7 (C); 128.9 (CH); 128.6 (CH); 127.7 (CH); 52.4 (CH_2); 47.9 (CH_2); 29.3 (CH_2); 29.2 (CH_2); 28.2 (CH_2); 27.0 (CH_2). IR ν 3307 (R-NH-R1); 3026 (Ar-H); 1121 (C-N-C); 697 (monosubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{24}\text{H}_{37}\text{N}_2$ ($\text{M}+\text{H}^+$) 353.2957; found 353.2966.

4.6.6. Synthesis of N1,N12-dibenzyldecane-1,12-diamine (6).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 97 mg of white solid (isolated yield: 79 %). ^1H NMR (CDCl_3) δ 7.31 (m, 10H, Ar-H); 3.84 (s, 4H, Ar- CH_2); 2.65 (t, 4H, $J = 7.20$ Hz, N- CH_2); 1.55 (m, 4H, C2-H and C11-H); 1.25 (m, 16H, C3-H to C10-H). ^{13}C NMR (CDCl_3) δ 136.7 (C); 128.9 (CH); 128.6 (CH); 127.7 (CH); 52.4 (CH_2); 47.9 (CH_2); 29.3 (CH_2); 29.2 (CH_2); 28.2 (CH_2); 27.0 (CH_2). IR ν 3307 (R-NH-R1); 3026 (Ar-H); 1121 (C-N-C); 697 (monosubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{26}\text{H}_{41}\text{N}_2$ ($\text{M}+\text{H}^+$) 381.3255; found 381.3264.

4.6.7. Synthesis of N1,N3-bis(4-methoxybenzyl)propane-1,3-diamine (7).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 132 mg of colourless oil (isolated yield: 78 %). ^1H NMR (CDCl_3) δ 7.21 (d, 4H, $J = 8.7$ Hz, C2'-H); 6.85 (d, 4H, $J = 8.7$ Hz, C3'-H); 3.79 (s, 6H, O- CH_3); 3.71 (s, 4H, Ar- CH_2); 2.68 (t, 4H, $J = 6.6$ Hz, N- CH_2); 1.71 (m, 2H, C2-H). ^{13}C NMR (CDCl_3) δ 158.6 (C); 132.7 (C); 129.3 (CH); 113.7 (CH); 55.2 (CH_3); 53.5 (CH_2); 47.9 (CH_2); 30.2 (CH_2). IR ν 3301 (R-NH-R1); 3062

(Ar-H); 1176 (C-N-C); 1243 and 1036 (Ar-O-C); 818 (disubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{19}\text{H}_{27}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 315.2073; found 315.2087.

4.6.8. Synthesis of N1,N4-bis(4-methoxybenzyl)butane-1,4-diamine (8).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 115 mg of white solid (isolated yield: 77 %). ^1H NMR (CDCl_3) δ 7.19 (d, 4H, $J= 8.7$ Hz, C2'-H); 6.83 (d, 4H, $J= 8.7$ Hz, C3'-H); 3.75 (s, 6H, O-CH₃); 3.68 (s, 4H, Ar-CH₂); 2.58 (t, 4H, $J= 6.6$ Hz, N-CH₂); 1.51 (m, 4H, C2-H and C3-H). ^{13}C NMR (CDCl_3) δ 158.6 (C); 132.4 (C); 129.3 (CH); 113.8 (CH); 55.2 (CH₃); 53.3 (CH₂); 49.1 (CH₂); 27.8 (CH₂). IR ν 3314(R-NH-R1); 2997 (Ar-H); 1246 and 1036 (Ar-O-C); 1174 (C-N-C); 818(disubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{20}\text{H}_{29}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 329.229; found 329.2233.

4.6.9. Synthesis of N1,N6-bis(4-methoxybenzyl)hexane-1,6-diamine (9).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 37 mg of white solid (isolated yield: 30 %). ^1H NMR (CDCl_3) δ 7.23 (d, 4H, $J= 8.7$ Hz, C2'-H); 6.86 (d, 4H, $J= 8.7$ Hz, C3'-H); 3.79 (s, 6H, -CH₃); 3.71 (s, 4H, Ar-CH₂); 2.59 (t, 4H, $J= 6.9$ Hz, N-CH₂); 1.48 (m, 4H, C2-H and C5-H); 1.33 (m, 4H, C3-H and C4-H). ^{13}C NMR (CDCl_3) δ 158.6 (C); 132.5 (C); 129.3 (CH); 113.8 (CH); 55.2 (CH₃); 53.4(CH₂); 49.2 (CH₂); 29.9 (CH₂); 27.3 (CH₂). IR ν 3296(R-NH-R1); 2957 (Ar-H); 1252 and 1033 (Ar-O-C); 1103 (C-N-C); 818 (disubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{22}\text{H}_{33}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 357.2542; found 357.2531.

4.6.10. Synthesis of N1,N8-bis(4-methoxybenzyl)octane-1,8-diamine (10).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 95 mg of white solid (isolated yield: 59 %). ^1H NMR (CDCl_3) δ 7.23 (d, 4H, $J= 8.7$ Hz, C2'-H); 6.85 (d, 4H, $J= 8.7$ Hz, C3'-H); 3.78 (s, 6H, O-CH₃); 3.71 (s, 4H, Ar-CH₂); 2.59 (t, 4H, $J= 6.9$ Hz, N-CH₂); 1.48 (m, 4H, C2-H and C7-H); 1.28 (m, 8H, C3-H to C6-H). ^{13}C NMR (CDCl_3) δ 158.6 (C); 132.4 (C); 129.3 (CH); 113.8 (CH); 55.2 (CH₃); 53.4 (CH₂); 49.3 (CH₂); 29.9 (CH₂); 29.5 (CH₂); 27.3 (CH₂). IR ν 3307 (R-NH-R1); 3026 (Ar-H); 1121 (C-N-C); 697 (monosubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{24}\text{H}_{37}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 385.2855; found: 385.2857.

4.6.11. Synthesis of N1,N10-bis(4-methoxybenzyl)decane-1,10-diamine (11).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 63 mg of white solid (isolated yield: 66 %). ^1H NMR (CDCl_3) δ 7.26 (d, 4H, J = 8.7 Hz, C2'-H); 6.86 (d, 4H, J = 8.7 Hz, C3'-H); 3.79 (s, 6H, O-CH₃); 3.75 (s, 4H, Ar-CH₂); 2.62 (t, 4H, J = 7.2 Hz, NH-CH₂); 1.52 (m, 4H, C2-H and C9-H); 1.26 (m, 12H, C3-H to C8-H). ^{13}C NMR (CDCl_3) δ 158.9 (C); 130.2 (C); 129.8 (CH); 113.9 (CH); 55.2 (CH₃); 52.4 (CH₂); 48.9 (CH₂); 29.4 (CH₂); 29.3 (CH₂); 28.9 (CH₂); 27.2 (CH₂). IR ν 3392(R-NH-R1); 1253 and 1033 (Ar-O-C); 1177 (C-N-C); 818 (disubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{26}\text{H}_{41}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 413.3168. found 413.3172.

4.6.12. Synthesis of N1,N12-bis(4-methoxybenzyl)dodecane-1,12-diamine (12).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 109 mg of white solid (isolated yield: 99 %). ^1H NMR (CDCl_3) δ 7.26 (d, 4H, J = 8.7 Hz, C2'-H); 6.86 (d, 4H, J = 8.7 Hz, C3'-H); 3.79 (s, 6H, O-CH₃); 3.75 (s, 4H, Ar-CH₂); 2.62 (t, 4H, J = 7.2 Hz, N-CH₂); 1.52 (m, 4H, C2-H and C11-H); 1.26 (m, 16H, C3-H to C10-H). ^{13}C NMR (CDCl_3) δ 158.9 (C); 130.2 (C); 129.8 (CH); 113.9 (CH); 55.2 (CH₃); 52.4 (CH₂); 48.9 (CH₂); 29.4 (CH₂); 29.3 (CH₂); 28.9 (CH₂); 27.2 (CH₂). IR ν 3392(R-NH-R1); 1253 and 1033 (Ar-O-C); 1177 (C-N-C); 818 (disubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{28}\text{H}_{45}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 441.3475; found 441.3462.

4.6.13. Synthesis of N1,N3-bis(4-(benzyloxy)benzyl)propane-1,3-diamine (13).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 170 mg of white solid (isolated yield: 67 %). ^1H NMR (CDCl_3) δ 7.41 (m, 10H, aromatics 4'-benzyloxy) 7.21 (d, 4H, J = 8.7 Hz, C2'-H); 6.93 (d, 4H, J = 8.7 Hz, C3'-H); 5.05 (s, 4H, Ar-CH₂-O); 3.71 (s, 4H, Ar-CH₂-N); 2.69 (t, 4H, J = 6.9 Hz, N-CH₂); 1.72 (m, 2H, C2). ^{13}C NMR (CDCl_3) δ 157.9 (C); 137.1 (C); 132.7 (C); 129.4 (CH); 128.6 (CH); 128.0 (CH); 127.5 (CH); 114.8 (CH); 70.1 (CH₂); 53.4 (CH₂); 47.9 (CH₂); 29.9 (CH₂). IR ν 3289 (R-NH-R1); 3032 (Ar-H); 1106 (C-N-C); 1239 and 1026 (Ar-O-C) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{31}\text{H}_{35}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 467.2699; found 467.2678.

4.6.14. Synthesis of N1,N4-bis(4-(benzyloxy)benzyl)butane-1,4-diamine (14).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 168 mg of white solid (isolated yield: 77 %). ^1H NMR (CDCl_3) δ 7.42 (m, 10H, aromatics 4'-benzyloxy); 7.22 (d, 4H, J = 8.7 Hz, C2'-H); 6.93 (d, 4H, J = 8.7 Hz, C3'-H);

5.05 (s, 4H, Ar-CH₂-O); 3.71 (s, 4H, Ar-CH₂-N); 2.62 (t, 4H, *J* = 6.6 Hz, N-CH₂); 1.53 (m, 4H, C2-H and C4-H). ¹³C NMR (CDCl₃) δ 157.8 (C); 137.1 (C); 132.9 (C); 129.3 (CH); 128.6 (CH); 127.9 (CH); 127.5 (CH); 114.8 (CH); 70.1 (CH₂); 53.4 (CH₂); 49.2 (CH₂); 27.9 (CH₂). IR ν 3294 (R-NH-R1); 3035 (Ar-H); 1167 (C-N-C); 1247 and 1015 (Ar-O-C); 814 (disubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₃₂H₃₇N₂O₂ (M+H⁺) 481.2855; found 481.2849.

4.6.15. Synthesis of N1,N6-bis(4-(benzyloxy)benzyl)hexane-1,6-diamine (15).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 155 mg of white solid (isolated yield: 86 %). ¹H NMR (CDCl₃) δ 7.38 (m, 10H, aromatics 4'-benzyloxy); 7.23 (d, 4H, *J* = 8.7 Hz, C2'-H); 6.93 (d, 4H, *J* = 8.7 Hz, C3'-H); 5.05 (s, 4H, Ar-CH₂-O); 3.72 (s, 4H, Ar-CH₂-N); 2.61 (t, 4H, *J* = 6.9 Hz, N-CH₂); 1.51 (m, 4H, C2-H and C5-H); 1.32 (m, 4H, C3-H and C4-H). ¹³C NMR (CDCl₃) δ 157.9 (C); 137.1 (C); 132.3 (C); 129.5 (CH); 128.6 (CH); 127.9 (CH); 127.5 (CH); 114.8 (CH); 70.0 (CH₂); 53.2 (CH₂); 49.1 (CH₂); 29.7 (CH₂); 27.2 (CH₂). IR ν 3293 (R-NH-R1); 3048 (Ar-H); 1104 (C-N-C); 1251 and 1016 (Ar-O-C); 815 (disubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₃₄H₄₁N₂O₂ (M+H⁺) 509.3168; found 509.3146.

4.6.16. Synthesis of N1,N8-bis(4-(benzyloxy)benzyl)octane-1,8-diamine (16).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 147 mg of white solid (isolated yield: 99 %). ¹H NMR (CDCl₃) δ 7.42 (m, 10H, aromatics 4'-benzyloxy); 7.23 (d, 4H, *J* = 8.7 Hz, C2'-H); 6.93 (d, 4H, *J* = 8.7 Hz, C3'-H); 5.05 (s, 4H, Ar-CH₂-O); 3.72 (s, 4H, Ar-CH₂-N); 2.60 (t, 4H, *J* = 7.2 Hz, N-CH₂); 1.49 (m, 4H, C2-H and C7-H); 1.29 (m, 8H, CH₂ C3-H to C6-H). ¹³C NMR (CDCl₃) δ 157.8 (C); 137.1 (C); 133.0 (C); 129.3 (CH); 128.6 (CH); 127.9 (CH); 127.5 (CH); 114.8 (CH); 70.0 (CH₂); 53.5 (CH₂); 49.4 (CH₂); 30.1 (CH₂); 29.5 (CH₂); 27.3 (CH₂). IR ν 3246 (R-NH-R1); 3035 (Ar-H); 1104 (C-N-C); 1249 and 1015 (Ar-O-C); 813 (disubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₃₆H₄₅N₂O₂ (M+H⁺) 537.3481; found 537.3477.

4.6.17. Synthesis of N1,N10-bis(4-(benzyloxy)benzyl)decane-1,10-diamine (17).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 79 mg of white solid (isolated yield: 60 %). ¹H NMR (CDCl₃) δ 7.42 (m, 10H, aromatics 4'-benzyloxy); 7.24 (d, 4H, *J* = 8.7 Hz, C2'-H); 6.93 (d, 4H, *J* = 8.7 Hz, C3'-H); 5.05 (s, 4H, Ar-CH₂-O); 3.72 (s, 4H, Ar-CH₂-N); 2.61 (t, 4H, *J* = 6.9 Hz, N-CH₂); 1.49 (m, 4H, C2-H

and C9-H); 1.27 (m, 12H, C3-H to C8-H). ^{13}C NMR (CDCl_3) δ 157.9 (C); 137.1 (C); 132.7 (C); 129.4 (CH), 128.6 (CH), 128.0 (CH), 127.5 (CH); 114.8 (CH); 70.0 (CH_2); 53.4 (CH_2); 49.4 (CH_2); 30.0 (CH_2); 29.5 (CH_2); 27.6 (CH_2). IR ν 3311 (R-NH-R1); 3049 (Ar-H); 1105 (C-N-C); 1250 and 1016 (Ar-O-C); 814 (disubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{38}\text{H}_{49}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 565.3794; found 565.3802.

4.6.18. Synthesis of N1,N12-bis(4-(benzyloxy)benzyl)dodecane-1,12-diamine (18).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 143 mg of white solid (isolated yield: 99 %). ^1H NMR (CDCl_3) δ 7.42 (m, 10H, aromatics 4'-benzyloxy); 7.24 (d, 4H, J = 8.7 Hz, C2'-H); 6.93 (d, 4H, J = 8.7 Hz, C3'-H); 5.05 (s, 4H, Ar- CH_2 -O); 3.72 (s, 4H, Ar- CH_2 -N); 2.61 (t, 4H, J = 6.9 Hz, N- CH_2); 1.49 (m, 4H, C2-H and C11-H); 1.27 (m, 16H, C3-H to C10-H). ^{13}C NMR (CDCl_3) δ 157.9 (C); 137.1 (C); 132.7 (C); 129.4 (CH); 128.6 (CH); 128.0 (CH); 127.5 (CH); 114.8 (CH); 70.0 (CH_2); 53.4 (CH_2); 49.4 (CH_2); 30.0 (CH_2); 29.5 (CH_2); 27.6 (CH_2). IR ν 3311 (R-NH-R1); 3049 (Ar-H); 1105 (C-N-C); 1250 and 1016 (Ar-O-C); 814 (disubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{38}\text{H}_{49}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 565.3794; found 565.3802.

4.6.19. Synthesis of 5,5'-((propane-1,3-diylbis(azanediyl)) bis(methylene))bis(2-methoxyphenol) (19).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 180 mg of white solid (isolated yield: 96 %). ^1H NMR (CDCl_3) δ 6.79 (m, 6H, aromatics); 3.87 (s, 6H, O- CH_3); 3.69 (s, 4H, Ar- CH_2); 2.73 (t, 4H, J = 6.6 Hz, N- CH_2); 1.74 (m, 2H, C2-H). ^{13}C NMR (CDCl_3) δ 146.8 (C); 146.7 (C); 134.1 (C); 118.9 (CH); 115.9 (CH); 112.4 (CH); 56.1 (CH_3); 53.1 (CH_2); 47.5 (CH_2); 30.0 (CH_2). IR (KBr) ν 3449 (R-NH-R1); 3029 (Ar-H); 2464 (Ar-O-H); 1280 and 1028 (Ar-O-C); 1132 (C-N-C) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{19}\text{H}_{27}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}^+$) 347.1971; found 347.1969.

4.6.20. Synthesis of 5,5'-((butane-1,4-diylbis(azanediyl)) bis(methylene))bis(2-methoxyphenol) (20).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 131 mg of white solid (isolated yield: 80 %). ^1H NMR (CDCl_3) δ 6.77 (m, 6H, aromatics); 3.87 (s, 6H, O- CH_3); 3.69 (s, 4H, Ar- CH_2); 2.62 (t, 4H, J = 6.6 Hz, N- CH_2);

1.56 (m, 4H, C2-H and C3-H). ^{13}C NMR (CDCl_3) δ 146.7 (C); 146.7 (C); 134.1 (C); 118.9 (CH); 115.8 (CH); 112.4 (CH); 56.1 (CH_3); 53.0 (CH_2); 49.0 (CH_2); 27.8 (CH_2). IR (KBr) ν 3441(R-NH-R1); 3040 (Ar-H); 2319 (Ar-O-H); 1255 and 1026 (Ar-O-C); 1138(C-N-C); 866 cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{20}\text{H}_{29}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}^+$) 361.2127; found 361.2121.

4.6.21. Synthesis of 5,5'-((hexane-1,6-diylbis(azanediyl)) bis(methylene))bis(2-methoxyphenol) (21).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 110 mg of white solid (isolated yield: 82 %). ^1H NMR (CDCl_3) δ 6.80 (m, 6H, aromatics); 3.86 (s, 6H, O- CH_3); 3.68 (s, 4H, Ar- CH_2); 2.59 (t, 4H, $J = 7.2\text{ Hz}$, N- CH_2); 1.47 (m, 4H, C2-H and C5-H); 1.30(m, 4H, C3-H and C4-H). ^{13}C NMR (CDCl_3) δ 146.7 (C); 146.7 (C); 134.1 (C); 118.9 (CH); 115.8 (CH); 112.4 (CH); 56.1 (CH_3); 53.1 (CH_2); 49.0 (CH_2); 29.9 (CH_2); 27.4 (CH_2). IR (KBr) ν 3465(R-NH-R1); 3031 (Ar-H); 2441 (Ar-O-H); 1286 and 1035 (Ar-O-C); 1131 (C-N-C) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{22}\text{H}_{33}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}^+$) 389.2440; found 389.2442.

4.6.22. Synthesis of 5,5'-((octane-1,8-diylbis(azanediyl)) bis(methylene))bis(2-methoxyphenol) (22).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 135 mg of white solid (isolated yield: 72 %). ^1H NMR (CDCl_3) δ 6.80 (m, 6H, aromatics); 3.86 (s, 6H, O- CH_3); 3.69 (s, 4H, Ar- CH_2); 2.58 (t, 4H, $J = 7.2\text{ Hz}$, N- CH_2); 1.47 (m, 4H, C2-H and C7-H); 1.25 (m, 8H, C3-H to C6-H). ^{13}C NMR (CDCl_3) δ 146.8 (C); 146.7 (C); 134.0 (C); 119.0 (CH); 115.9 (CH); 112.4 (CH); 56.1 (CH_3); 53.1 (CH_2); 49.0 (CH_2); 29.9 (CH_2); 29.5 (CH_2); 27.3 (CH_2). IR (KBr) ν 3447(R-NH-R1); 2991 (Ar-H); 2445 (Ar-O-H); 1282 and 1033 (Ar-O-C); 1131(C-N-C); 855 (trisubstituted aromatic) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{24}\text{H}_{37}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}^+$) 417.2753; found 417.2740.

4.6.23. Synthesis of 5,5'-((decane-1,10-diylbis(azanediyl)) bis(methylene)) bis(2-methoxyphenol) (23).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 74 mg of white solid (isolated yield: 72 %). ^1H NMR (CDCl_3) δ 6.82 (m, 6H, aromatics); 3.86 (s, 6H, O- CH_3); 3.70 (s, 4H, Ar- CH_2); 2.60 (t, 4H, $J = 7.2\text{ Hz}$, N- CH_2); 1.48 (m, 4H, C2-H and C9-H); 1.25 (m, 12H, CH_2 C3- H to C8-H). ^{13}C NMR (CDCl_3) δ 146.7 (C); 146.7

(C); 134.1 (C); 118.9 (CH); 115.8 (CH); 112.4 (CH); 56.1 (CH₃); 53.1 (CH₂); 49.0 (CH₂); 29.9 (CH₂); 29.5 (CH₂); 27.6 (CH₂). IR (KBr) ν 3418 (R-NH-R1); 3033 (Ar-H); 2444 (Ar-O-H); 1282 and 1033 (Ar-O-C); 1131(C-N-C); 873 (trisubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₂₄H₄₁N₂O₄ (M+H⁺) 445.3066; found 445.3050.

4.6.24. Synthesis of 5,5'-((dodecane-1,12-diylbis(azanediyl)) bis(methylene))bis(2-methoxyphenol) (24).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 86 mg of white solid (isolated yield: 46 %). ¹H NMR (CDCl₃) δ 6.82 (m, 6H, aromatics); 3.86 (s, 6H, O-CH₃); 3.70 (s, 4H, Ar-CH₂); 2.60 (t, 4H, *J* = 7.2 Hz, N-CH₂); 1.48 (m, 4H, C2-H and C11-H); 1.25 (m, 16H, CH₂ C3- H to C12-H). ¹³C NMR (CDCl₃) δ 146.7 (C); 146.7 (C); 134.1 (C); 118.9 (CH); 115.8 (CH); 112.4 (CH); 56.1 (CH₃); 53.1 (CH₂); 49.0 (CH₂); 29.9 (CH₂); 29.5 (CH₂); 27.6 (CH₂). IR (KBr) ν 3418(R-NH-R1); 3033 (Ar-H); 2444 (Ar-O-H); 1282 and 1033 (Ar-O-C); 1131(C-N-C); 873 (trisubstituted aromatic) cm⁻¹.

4.6.25. Synthesis of N1,N3-bis(4-(benzyloxy)-3-methoxybenzyl)propane-1,3-diamine (25).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 133 mg of white solid (isolated yield: 50 %). ¹H NMR (CDCl₃) δ 7.37 (m, 10H, aromatics 4'-benzyloxy); 6.82 (m, 6H, aromatics); 5.13 (s, 4H, Ar-CH₂-O); 3.87 (s, 6H, O-CH₃); 3.70 (s, 4H, Ar-CH₂-N); 2.69 (t, 4H, *J* = 6.6 Hz, N-CH₂-); 1.72 (m, 2H, C2-H). ¹³C NMR (CDCl₃) δ 149.7 (C); 147.2 (C); 137.3 (C); 133.8 (C); 128.5 (CH); 127.8 (CH); 127.3 (CH); 120.2 (CH); 114.1 (CH); 112.0 (CH); 71.1 (CH₂); 56.0 (CH₃); 53.9 (CH₂); 48.0 (CH₂); 30.2 (CH₂). IR ν 3302 (R-NH-R1); 3063 (Ar-H); 1264 and 1026 (Ar-O-C); 1139 (C-N-C) cm⁻¹. ESI-HRMS: calculated mass for C₃₃H₃₉N₂O₄ (M+H⁺) 527.2910; found 527.2886.

4.6.26. Synthesis of N1,N4-bis(4-(benzyloxy)-3-methoxybenzyl)butane-1,4-diamine (26).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 205 mg of white solid (isolated yield: 84 %). ¹H NMR (CDCl₃) δ 7.39 (m, 10H, aromatics 4'-benzyloxy); 6.82 (m, 6H, aromatics); 5.13 (s, 4H, Ar-CH₂-O); 3.89 (s, 6H, O-CH₃); 3.70 (s, 4H, Ar-CH₂-N); 2.64 (t, 4H, *J* = 6.6 Hz, N-CH₂); 1.56 (m, 4H, C2-H and C3-H). ¹³C NMR (CDCl₃) δ 149.7 (C); 147.2 (C); 137.3 (C); 133.2 (C); 128.5 (CH), 127.8 (CH), 127.3 (CH); 120.3 (CH); 114.0 (CH); 112.0 (CH); 71.1 (CH₂); 56.0 (CH₃); 53.6 (CH₂); 49.1 (CH₂); 27.7 (CH₂). IR

ν 3299 (R-NH-R1); 3066 (Ar-H); 1261 and 1032 (Ar-O-C); 1131 (C-N-C) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{34}\text{H}_{41}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 541.3066; found 541.3045.

4.6.27. Synthesis of N1,N6-bis(4-(benzyloxy)-3-methoxybenzyl)hexane-1,6-diamine (27).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 159 mg of white solid (isolated yield: 81 %). ^1H NMR (CDCl_3) δ 7.40 (m, 10H, aromatics 4'-benzyloxy); 6.84 (m, 6H, aromatics); 5.13 (s, 4H, Ar-CH₂-O); 3.89 (s, 6H, O-CH₃); 3.71 (s, 4H, Ar-CH₂-N); 2.61 (t, 4H, $J = 7.2$ Hz, N-CH₂); 1.51 (m, 4H, C2-H and C5-H); 1.33 (m, 4H, C3-H and C4-H). ^{13}C NMR (CDCl_3) δ 149.7 (C); 147.6 (C); 137.3 (C); 133.4 (C); 128.5 (CH); 127.8 (CH); 127.3 (CH); 120.3 (CH); 114.0 (CH); 112.0 (CH); 71.1 (CH₂); 56.0 (CH₃); 53.7 (CH₂); 49.3 (CH₂); 29.8 (CH₂); 27.3 (CH₂). IR ν 3409 (R-NH-R1); 1264 and 1004 (Ar-O-C); 1145 (C-N-C) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{36}\text{H}_{45}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}^+$) 569.3379; found 569.3380.

4.6.28. Synthesis of N1,N8-bis(4-(benzyloxy)-3-methoxybenzyl)octane-1,8-diamine (28).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 155 mg of white solid (isolated yield: 88 %). ^1H NMR (CDCl_3) δ 7.39 (m, 10H, aromatics 4'-benzyloxy); 6.84 (m, 6H, aromatics); 5.14 (s, 4H, Ar-CH₂-O); 3.89 (s, 6H, O-CH₃); 3.71 (s, 4H, Ar-CH₂-N); 2.61 (t, 4H, $J = 7.2$ Hz, N-CH₂); 1.46 (m, 4H, C2-H and C7-H); 1.29 (m, 8H, C3-H to C6-H). ^{13}C NMR (CDCl_3) δ 149.7 (C); 147.2 (C); 137.3 (C); 133.2 (C); 128.5 (CH); 127.8 (CH); 127.3 (CH); 120.3 (CH); 114.0 (CH); 112.0 (CH); 71.1 (CH₂); 56.0 (CH₃); 53.7 (CH₂); 49.3 (CH₂); 29.8 (CH₂); 29.4 (CH₂); 27.2 (CH₂). IR ν 3410 (R-NH-R1); 3006 (Ar-H); 1259 and 1029 (Ar-O-C); 1162 (C-N-C) cm^{-1} . ESI-HRMS: calculated mass for $\text{C}_{38}\text{H}_{49}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}^+$) 597.3692; found 597.3717.

4.6.29. Synthesis of N1,N10-bis(4-(benzyloxy)-3-methoxybenzyl)decane-1,10-diamine (29).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 130 mg of white solid (isolated yield: 89 %). ^1H NMR (CDCl_3) δ 7.40 (m, 10H, aromatics 4'-benzyloxy); 6.81 (m, 6H, aromatics); 5.14 (s, 4H, Ar-CH₂-O); 3.89 (s, 6H, O-CH₃); 3.71 (s, 4H, Ar-CH₂-N); 2.61 (t, 4H, N-CH₂); 1.51 (m, 4H, C2-H and C9-H); 1.27 (m, 12H, C3-H to C8-H). ^{13}C NMR (CDCl_3) δ 149.7 (C); 147.2 (C); 137.3 (C); 133.6 (C); 128.5 (CH); 127.8 (CH); 127.3 (CH); 120.3 (CH); 114.0 (CH); 112.0 (CH); 71.1 (CH₂); 56.0 (CH₃); 53.8 (CH₂); 49.4 (CH₂);

30.0 (CH₂); 29.5 (CH₂); 27.4 (CH₂). IR ν 3422 (R-NH-R1); 1263 and 1029 (Ar-O-C); 1140 (C-N-C) cm⁻¹. ESI-HRMS: calculated mass for C₄₀H₅₃N₂O₄ (M+H⁺) 625.4005; found 625.4030.

4.6.30. Synthesis of N1,N12-bis(4-(benzyloxy)-3-methoxybenzyl) dodecane-1,12-diamine (30).

Following the general reaction conditions for the disubstituted diamines synthesis, the reaction was worked up and purified to afford 135 mg of white solid (isolated yield: 90 %). ¹H NMR (CDCl₃) δ 7.40 (m, 10H, aromatics 4'-benzyloxy); 6.81 (m, 6H, aromatics); 5.14 (s, 4H, Ar-CH₂-O); 3.89 (s, 6H, O-CH₃); 3.71 (s, 4H, Ar-CH₂-N); 2.61 (t, 4H, N-CH₂); 1.51 (m, 4H, C2-H and C11-H); 1.27 (m, 16H, C3-H to C10-H). ¹³C NMR (CDCl₃) δ 149.7 (C); 147.2 (C); 137.3 (C); 133.6 (C); 128.5 (CH); 127.8 (CH); 127.3 (CH); 120.3 (CH); 114.0 (CH); 112.0 (CH); 71.1 (CH₂); 56.0 (CH₃); 53.8 (CH₂); 49.4 (CH₂); 30.0 (CH₂); 29.5 (CH₂); 27.4 (CH₂). IR ν 3422 (R-NH-R1); 1263 and 1029 (Ar-O-C); 1140 (C-N-C) cm⁻¹. ESI-HRMS: calculated mass for C₄₂H₅₇N₂O₄ (M+H⁺) 653.4313; found 653.4313.

4.7. General procedure for N,N,N',N'-diamines tetrasubstituted synthesis

To a solution of aldehyde (4.5 eq.) and diamine (1 eq) in anhydrous DCM, NaBH(AcO)₃ (7.2 eq.) and acetic acid (6.4 eq.) were added at room temperature. The reaction was stirred under inert atmosphere for 24 hours and then was quenched with a 10% NaHCO₃. Solution. Phases were separated and the aqueous phase extracted with DCM (3 x 20 mL). Combined organic extracts were dried with sodium sulphate and evaporated. Products were purified by flash chromatography.

4.7.1 Synthesis of N1,N1,N3,N3-tetrabenzyl-propane-1,3-diamine (31).

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 29 mg of white solid (isolated yield: 17 %). ¹H NMR (CDCl₃) δ 7.28 (m, 20H, aromatics); 3.51 (s, 8H, Ar-CH₂); 2.42 (t, 4H, *J*= 6.9 Hz, N-CH₂); 1.73 (m, 2H, C2-H). ¹³C NMR (CDCl₃) δ 139.7 (C); 128.9 (CH); 128.2 (CH); 126.8 (CH); 58.4 (CH₂); 51.6 (CH₂); 24.5 (CH₂). IR ν 3026 (Ar-H); 1127 (C-N); 697 (monosubstituted aromatic) cm⁻¹. ESI-HRMS: calculated mass for C₃₁H₃₅N₂ (M+H⁺) 435.2800; found 435.2781.

4.7.2 Synthesis of N1,N1,N4,N4-tetrabenzylbutane-1,4-diamine (32)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 42 mg of white solid (isolated yield: 26 %). ¹H NMR (CDCl₃) δ 7.31 (m, 20H, aromatics); 3.50 (s, 8H, Ar-CH₂); 2.35 (t, 4H, *J*= 4.8 Hz, N-CH₂); 1.49 (m, 4H, C2-H and C3-H). ¹³C NMR (CDCl₃) δ 140.0 (C); 128.8 (CH); 128.2 (CH); 126.07 (CH); 58.3 (CH₂); 53.4

(CH₂); 24.8 (CH₂). IR ν 3028 (Ar-H); 1123 (C-N); 693 (monosubstituted aromatic) cm⁻¹. ESI-HRMS: mass calculated for C₃₂H₃₇N₂ (M+H⁺) 449.2957; found 449.2964.

4.7.3 Synthesis of N1,N1,N6,N6-tetrabenzylhexane-1,6-diamine (33)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 27 mg of white solid (isolated yield: 17 %). ¹H NMR (CDCl₃) δ 7.31 (m, 20H, aromatics); 3.53 (s, 8H, Ar-CH₂); 2.36 (t, 4H, *J*=6.9 Hz, N-CH₂); 1.45 (m, 4H, C2-H and C5-H); 1.17 (m, 4H, C3-H and C4-H). ¹³C NMR (CDCl₃) δ 140.1 (C); 128.8 (CH); 128.1 (CH); 126.7 (CH); 58.3 (CH₂); 53.4 (CH₂); 27.1 (CH₂); 27.0 (CH₂). IR ν 3026 (Ar-H); 1120 (C-N); 698 (monosubstituted aromatic) cm⁻¹. ESI-HRMS: mass calculated for C₃₄H₄₁N₂ (M+H⁺) 477.3270; found 477.3273.

4.7.4 Synthesis of N1,N1,N8,N8-tetrabenzyl-octane-1,8-diamine (34)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 28 mg of white solid (isolated yield: 27 %). ¹H NMR (CDCl₃) δ 7.32 (m, 20H, aromatics); 3.54 (s, 8H, Ar-CH₂); 2.39 (t, 4H, *J*= 6.9, N-CH₂); 1.47 (m, 4H, C2-H and C7-H); 1.22 (m, 8H, C3-H to C6-H). ¹³C NMR (CDCl₃) δ 140.1 (C); 128.8 (CH); 128.1 (CH); 126.7 (CH); 58.3 (CH₂); 53.4 (CH₂); 29.5 (CH₂); 27.3 (CH₂); 27.0 (CH₂). IR ν 3026 (Ar-H); 1126 (C-N); 697 (monosubstituted aromatic) cm⁻¹.

4.7.5 Synthesis of N1,N1,N10,N10-tetrabenzyldecane-1,10-diamine (35)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 26 mg of white solid (isolated yield: 28 %). ¹H NMR (CDCl₃) δ 7.32 (m, 20H, aromatics); 3.54 (s, 8H, Ar-CH₂); 2.39 (t, 4H, *J*=7.2 Hz, N-CH₂); 1.48 (m, 4H, C2-H and C9-H); 1.17 (s, 12H, C3-H to C8-H). ¹³C NMR (CDCl₃) δ 140.1 (C); 128.8 (CH); 128.1 (CH); 126.7 (CH); 58.3 (CH₂); 53.4 (CH₂); 29.6 (CH₂); 29.5 (CH₂); 27.3 (CH₂); 27.0 (CH₂). ESI-HRMS: calculated mass for C₃₈H₄₉N₂ (M+H⁺) 533.3892, found 533.3896.

4.7.6 Synthesis of N1,N1,N3,N3-tetrakis(4-methoxybenzyl)propane-1,3-diamine (36)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 194 mg of yellowish oil (isolated yield: 30%). ¹H NMR (CDCl₃) δ 7.16 (d, 8H, *J*=8.7 Hz, aromatics); 6.81 (d, 8H, *J* = 8.7 Hz, aromatics); 3.79 (s, 12H, O-CH₃); 3.40 (s, 8H, Ar-CH₂); 2.36 (t, 4H, *J*= 6.9 Hz, N-CH₂); 1.67 (m, 2H, C2). ¹³C NMR (CDCl₃) δ 158.5 (C); 132.0

(C); 130.0 (CH); 113.5 (CH); 57.5 (CH₂); 55.2 (CH₃); 51.3 (CH₂); 24.5 (CH₂). IR ν 2997 (Ar-H); 1247 and 1034 (Ar-O-C); 1179 (C-N) cm⁻¹. ESI-HRMS: calculated mass for C₃₅H₄₃N₂O₄ (M+H⁺) 555.3223; found 555.3235.

4.7.7 Synthesis of N1,N1,N4,N4-tetrakis(4-methoxybenzyl)butane-1,4-diamine (37)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 45 mg of white solid (isolated yield: 25%). ¹H NMR (CDCl₃) δ 7.22 (d, 8H, *J* = 8.7 Hz, aromatics); 6.83(d, 8H, *J* = 8.7 Hz, aromatics); 3.79 (s, 12H, -O-CH₃); 3.42 (s, 8H, Ar-CH₂); 2.31 (t, 4H, N-CH₂); 1.44 (m, 4H, C2-H and C3-H). ¹³C NMR (CDCl₃) δ 158.5 (C); 132.1 (C); 129.9 (CH); 113.5 (CH); 57.4 (CH₂); 55.3 (CH₃); 51.3 (CH₂); 24.8 (CH₂). IR ν 2998 (Ar-H); 1247 and 1037 (Ar-O-C); 1178 (C-N) cm⁻¹. ESI-HRMS: mass calculated for C₃₆H₄₅N₂O₄ (M+H⁺) 569.3379; found 569.3385.

4.7.8 Synthesis of N1,N1,N6,N6-tetrakis(4-methoxybenzyl)hexane-1,6-diamine (38)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 21 mg of white solid (isolated yield: 10%). ¹H NMR (CDCl₃) δ 7.24 (d, 8H, *J* = 8.7 Hz, aromatics); 6.83(d, 8H, *J* = 8.7 Hz, aromatics); 3.79 (s, 12H, O-CH₃); 3.45 (s, 8H, Ar-CH₂); 2.33 (t, 4H, *J* = 7.2 Hz, N-CH₂); 1.46 (m, 4H, C2-H and C5-H); 1.16 (m, 4H, C3-H and C4-H). ¹³C NMR (CDCl₃) δ 158.4 (C); 132.0 (C); 129.8 (CH); 113.5 (CH); 57.3 (CH₂); 55.2 (CH₃); 51.1 (CH₂); 27.2 (CH₂); 27.0 (CH₂). IR ν 3032 (Ar-H); 1247 and 1036 (Ar-O-C); 1170 (C-N) cm⁻¹. ESI-HRMS: mass calculated for C₃₈H₄₉N₂O₄ (M+H⁺) 597.3692; found 597.3718.

4.7.9 Synthesis of N1,N1,N8,N8-tetrakis(4-methoxybenzyl)octane-1,8-diamine (39)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 20 mg of white solid (isolated yield: 16%). ¹H NMR (CDCl₃) δ 7.25 (d, 8H, *J* = 8.7 Hz, aromatics); 6.84 (d, 8H, *J* = 8.7 Hz, aromatics); 3.79 (s, 12H, O-CH₃); 3.46 (s, 8H, Ar-CH₂); 2.37 (t, 4H, *J* = 7.2 Hz, N-CH₂); 1.46 (m, 4H, C2-H and C7-H); 1.16 (s, 8H, C3-H to C6-H). ¹³C NMR (CDCl₃) δ 158.4 (C); 132.1 (C); 129.9 (CH); 113.5 (CH); 57.4 (CH₂); 55.2 (CH₃); 53.1 (CH₂); 29.6 (CH₂); 27.3 (CH₂); 27.3 (CH₂). ESI-HRMS: mass calculated for C₄₀H₅₃N₂O₄ (M+H⁺) 625.4005; found 625.4036.

4.7.10 Synthesis of N1,N1,N10,N10-tetrakis(4-methoxybenzyl)decane-1,10 diamina (40)

Following the general reaction conditions for the tetrasubstituted diamines synthesis, the reaction was worked up and purified to afford 21 mg of white solid (isolated yield: 28%). ^1H NMR (CDCl_3) δ 7.25 (d, 8H, $J = 8.7$ Hz, aromatics); 6.84 (d, 8H, $J = 8.7$ Hz, aromatics); 3.79 (s, 12H, O-CH₃); 3.46 (s, 8H, Ar-CH₂); 2.36 (t, 4H, $J = 7.5$ Hz, N-CH₂); 1.47 (m, 4H, C2-H and C9-H); 1.18 (s, 12H, CH₂, C3-H to C8-H). ^{13}C NMR (CDCl_3) δ 158.4 (C); 132.0 (C); 129.9 (CH); 113.5 (CH); 57.4 (CH₂); 55.2 (CH₃); 53.0 (CH₂); 29.8 (CH₂); 29.5 (CH₂); 27.3 (CH₂); 26.9 (CH₂). ESI-HRMS: mass calculated for $\text{C}_{42}\text{H}_{57}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}^+$) 653.4318; found 653.4340.

4.8 Computational methods

The geometries of the products were optimized using B3LYP functional⁵⁴⁻⁵⁶ together with 6-311++G(d,p) basis set. The B3LYP has shown to be appropriate to obtain reliable geometries and for describing electronic density surfaces. All calculations were performed with the Gaussian 09 package.⁵⁷

Acknowledgments

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Competing interests

The authors declare that they have no competing interest.

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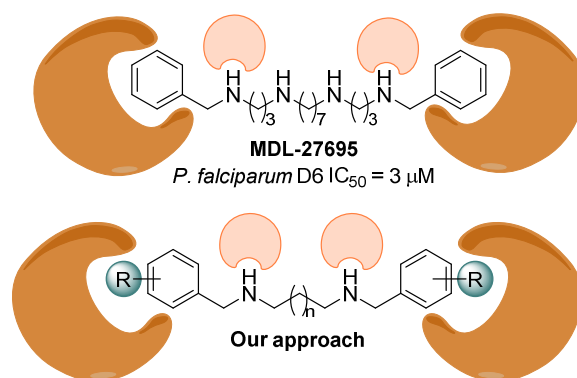
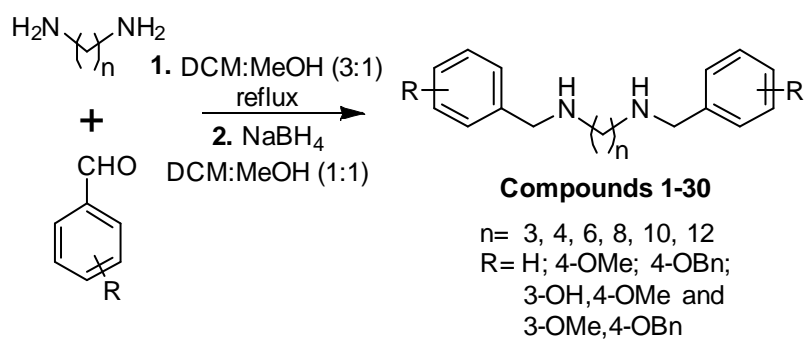


Figure 1. Structure of MDL-27695 and our strategy.



Scheme 1. Synthesis of *N,N'*-derivatives.

Table 1. Anti-apicomplexan activity, cytotoxicity and cluster family of N, N'-substituted diamines.

Compound	n	R	Yield*(%)	<i>P. falciparum</i> D6 IC ₅₀ (μM)	SI ^a	<i>P. falciparum</i> W2 IC ₅₀ (μM)	SI ^a	<i>T. gondii</i> Growth inhibition at 1 μM ^b	Cytotoxicity Vero cells IC ₅₀ (μM) ^c	Cluster
1	3	H	79	2.35	>7.9	2.63	>7.1	+	NC	A
2	4	H	73	2.98	>5.9	2.49	>7.1	+	NC	A
3	6	H	46	2.09	>7.7	2.09	>7.7	+	NC	B
4	8	H	94	0.37	>39.7	0.37	>39.7	+	NC	B
5	10	H	89	0.48	>28.0	0.28	>47.6	+++	NC	B
6	12	H	79	0.97	>12.9	1.02	>12.2	+	NC	B
7	3	4-OMe	78	3.18	>4.8	5.41	>2.8	++	NC	C
8	4	4-OMe	77	4.57	>3.2	6.69	>2.2	+	NC	D
9	6	4-OMe	30	3.08	2.4	3.36	2.5	+	8.32	E
10	8	4-OMe	59	1.48	>8.4	1.69	>7.3	+	NC	F
11	10	4-OMe	66	0.60	>19.0	0.67	>17.0	++	NC	F
12	12	4-OMe	99	1.58	>6.8	1.86	>5.8	+++	NC	F
13	3	4-OBn	67	1.22	4.9	1.43	4.2	+++	6.00	G
14	4	4-OBn	77	2.08	2.8	3.12	1.9	++	5.83	H
15	6	4-OBn	86	0.39	>24.0	0.29	>32.2	+	NC	I
16	8	4-OBn	99	3.72	>2.4	4.09	>2.2	+	NC	J
17	10	4-OBn	60	2.47	>3.4	3.50	>2.4	+++	NC	J
18	12	4-OBn	99	8.00	>1.2	8.00	>1.2	ND	NC	J
19	3	3-OH, 4-OMe	96	>8.00	-	>8.00	-	ND	NC	C
20	4	3-OH, 4-OMe	80	>8.00	-	>8.00	-	+++	NC	D
21	6	3-OH, 4-OMe	82	>8.00	-	>8.00	-	++	NC	E
22	8	3-OH, 4-OMe	78	4.80	>2.38	5.52	>2.1	+	NC	F
23	10	3-OH, 4-OMe	72	1.97	>5.41	1.88	>5.7	+	NC	F
24	12	3-OH, 4-OMe	46	1.97	>5.10	2.88	>3.5	+	NC	F
25	3	3-OMe, 4-OBn	50	0.42	15.9	0.47	14.1	++++	6.65	G
26	4	3-OMe, 4-OBn	84	0.33	>27.4	0.49	>18.4	++	NC	H
27	6	3-OMe, 4-OBn	81	0.26	>31.7	0.44	>19.0	+	NC	I
28	8	3-OMe, 4-OBn	88	0.31	14.6	0.43	10.5	-	4.52	J
29	10	3-OMe, 4-OBn	89	1.12	>6.8	1.05	>7.2	+	NC	J
30	12	3-OMe, 4-OBn	99	1.28	>5.67	1.37	>5.3	+	NC	J
31	3	H tetrasubstituted	17	>8.00	-	>8.00	-	ND	NC	K
32	4	H tetrasubstituted	26	>8.00	-	>8.00	-	ND	NC	K
33	6	H tetrasubstituted	17	>8.00	-	>8.00	-	ND	NC	L
34	8	H tetrasubstituted	27	>8.00	-	>8.00	-	ND	NC	L
35	10	H tetrasubstituted	28	>8.00	-	>8.00	-	ND	NC	L
36	3	4-OMe Tetrasubst.	30	>8.00	-	>8.00	-	ND	NC	K
37	4	4-OMe Tetrasubst.	25	>8.00	-	>8.00	-	ND	NC	K
38	6	4-OMe Tetrasubst.	10	>8.00	-	>8.00	-	ND	NC	L
39	8	4-OMe Tetrasubst.	16	>8.00	-	>8.00	-	ND	NC	L
40	10	4-OMe Tetrasubst.	28	>8.00	-	>8.00	-	ND	NC	L
CQ				0.083		0.422				
Art				0.094		0.094				
Pyr								-		

NC= not cytotoxic at the maximum concentration tested (4.46 ug/mL) CQ= Chloroquine Art= Artemisinin
Pyr=pyremethamine.

a-SI (Selectivity Index: IC₅₀ Vero Cells / IC₅₀ Parasites)

b Scale: ++++ total growth inhibition of parasite with fibroblast integrity, +++ Some tachyzoite growth but approximately at 75 % of control with fibroblast integrity, ++ correspond at 50% of parasite growth, + at 25% and - no growth inhibition.

c-Tested at a maximum concentration of 8 μM

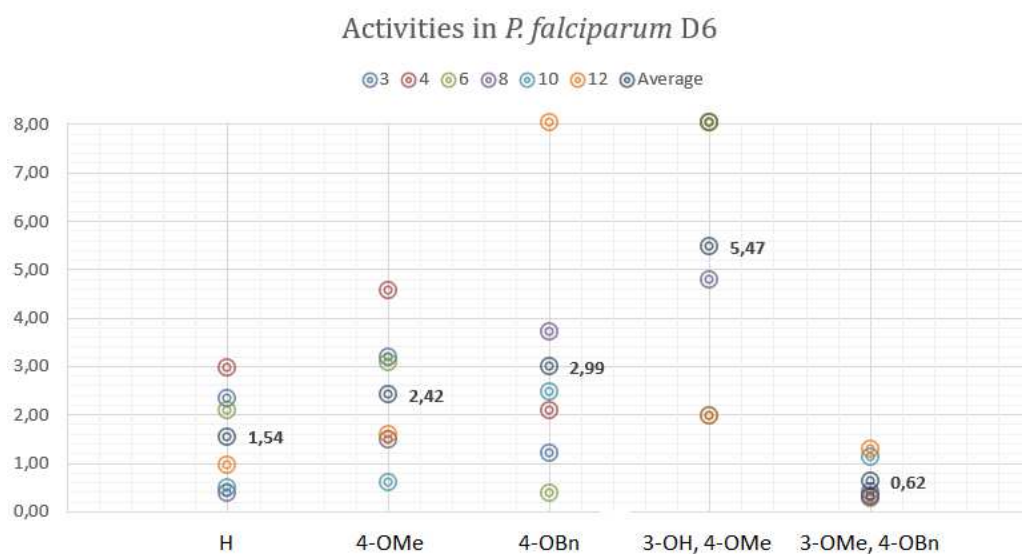


Figure 2. Antimalarial activity (D6 Clone) by substituent. The average value of each subset is shown.

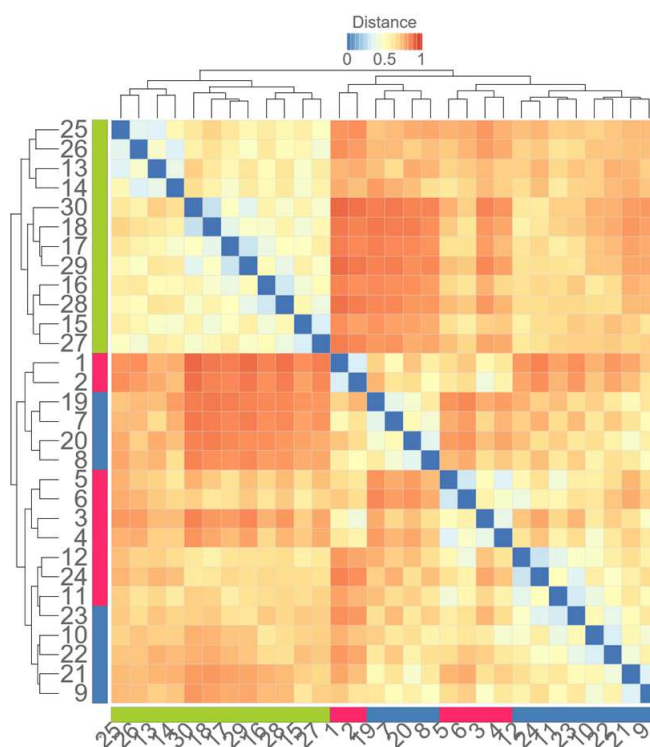


Figure 3. Cluster and dendrogram analysis of N,N'-di substituted diamine library.

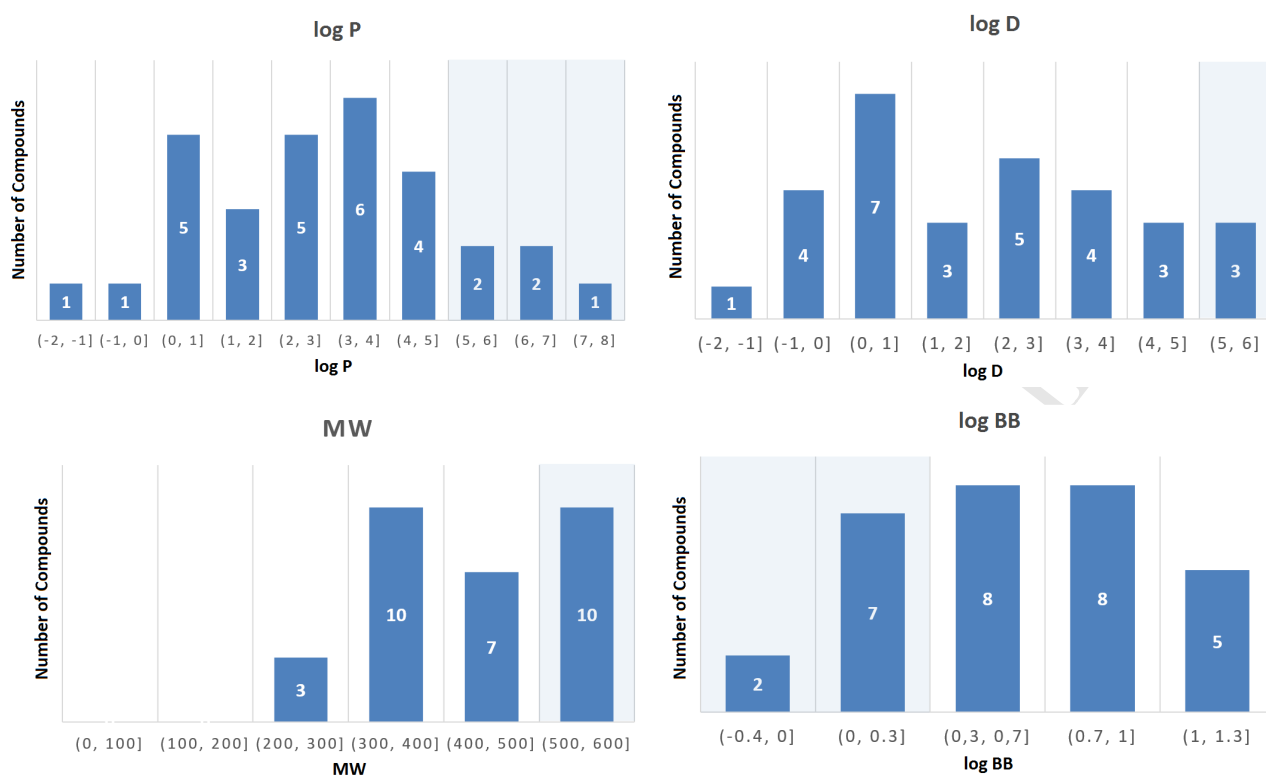


Figure 4. Distribution of compounds of synthetic library vs. physicochemical properties.

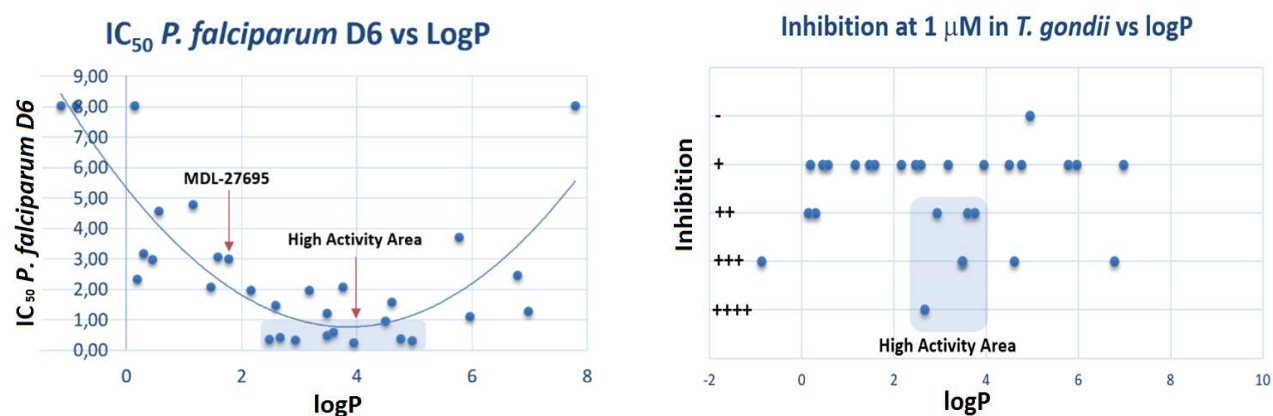
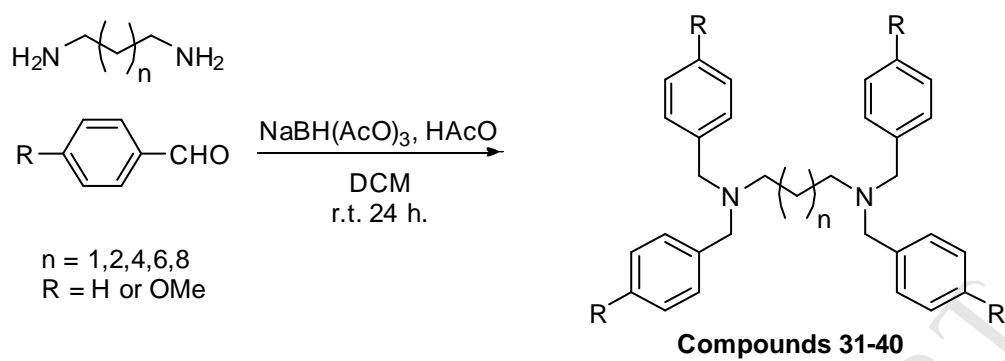


Figure 5. Parasitic activities (*P. falciparum* and *T. gondii*) vs. logP.

Table 2. Physicochemical properties of N, N'-substituted diamines.

Compound	n	R	MW	LogP	LogD	LogBB	TPSA	Volume
1	3	H	254.377	2.538	0.46	0.373	24.06	264.27
2	4	H	268.404	2.994	-0.88	0.436	24.06	281.07
3	6	H	296.458	3.906	-0.01	0.584	24.06	314.67
4	8	H	324.512	4.818	0.88	0.735	24.06	348.27
5	10	H	352.566	5.730	1.77	0.851	24.06	381.88
6	12	H	380.620	6.642	2.66	1.005	24.06	415.48
7	3	4-OMe	314.429	2.807	0.26	0.119	42.52	315.36
8	4	4-OMe	328.456	3.261	-1.09	0.193	42.52	332.16
9	6	4-OMe	356.510	4.095	-0.16	0.670	42.52	365.76
10	8	4-OMe	384.564	4.930	0.73	0.747	42.52	399.37
11	10	4-OMe	412.618	5.764	1.62	0.929	42.52	432.97
12	12	4-OMe	440.672	6.599	2.51	1.082	42.52	466.57
13	3	4-OBn	466.625	6.272	3.64	0.300	42.52	458.66
14	4	4-OBn	480.652	6.726	2.35	0.370	42.52	475.46
15	6	4-OBn	508.706	7.560	3.23	0.812	42.52	509.06
16	8	4-OBn	536.760	8.395	4.12	0.944	42.52	542.66
17	10	4-OBn	564.814	9.230	5.01	1.098	42.52	576.27
18	12	4-OBn	592.868	10.064	5.89	1.252	42.52	609.87
19	3	3-OH, 4-OMe	346.427	2.028	0.23	-0.308	82.98	331.39
20	4	3-OH, 4-OMe	360.454	2.482	-0.83	-0.294	82.98	348.19
21	6	3-OH, 4-OMe	388.508	3.316	0.05	0.142	82.98	381.80
22	8	3-OH, 4-OMe	416.562	4.151	0.94	0.297	82.98	415.40
23	10	3-OH, 4-OMe	444.616	4.985	1.83	0.448	82.98	449.01
24	12	3-OH, 4-OMe	472.670	5.820	2.71	0.604	82.98	482.61
25	3	3-OMe, 4-OBn	526.680	6.030	3.48	0.116	60.98	509.75
26	4	3-OMe, 4-OBn	540.704	6.473	2.26	0.130	60.98	526.55
27	6	3-OMe, 4-OBn	568.758	7.308	3.12	0.593	60.98	560.15
28	8	3-OMe, 4-OBn	596.812	8.142	4.02	0.776	60.98	593.76
29	10	3-OMe, 4-OBn	624.866	8.977	4.95	0.942	60.98	627.36
30	12	3-OMe, 4-OBn	652.920	8.977	5.92	1.057	60.98	660.96
31	3	H TetraSubst.	434.63	6.24	4.84	NC	6.48	441.45
32	4	H TetraSubst.	448.65	6.51	3.32	NC	6.48	458.25
33	6	H TetraSubst.	476.71	7.52	4.20	NC	6.48	491.86
34	8	H TetraSubst.	504.76	8.42	5.09	NC	6.48	525.46
35	10	H TetraSubst.	532.82	8.93	5.97	NC	6.48	559.06
36	3	4-OMe TetraS.	498.62	4.32	3.95	NC	87.39	473.52
37	4	4-OMe TetraS.	512.65	4.59	2.61	NC	87.39	490.32
38	6	4-OMe TetraS.	540.70	5.60	3.49	NC	87.39	523.93
39	8	4-OMe TetraS.	568.76	6.61	4.38	NC	87.39	557.53
40	12	4-OMe TetraS.	596.81	7.62	5.27	NC	87.39	591.13

NC= not calculated



Scheme 2. Synthesis of *N,N,N',N'*-derivatives.

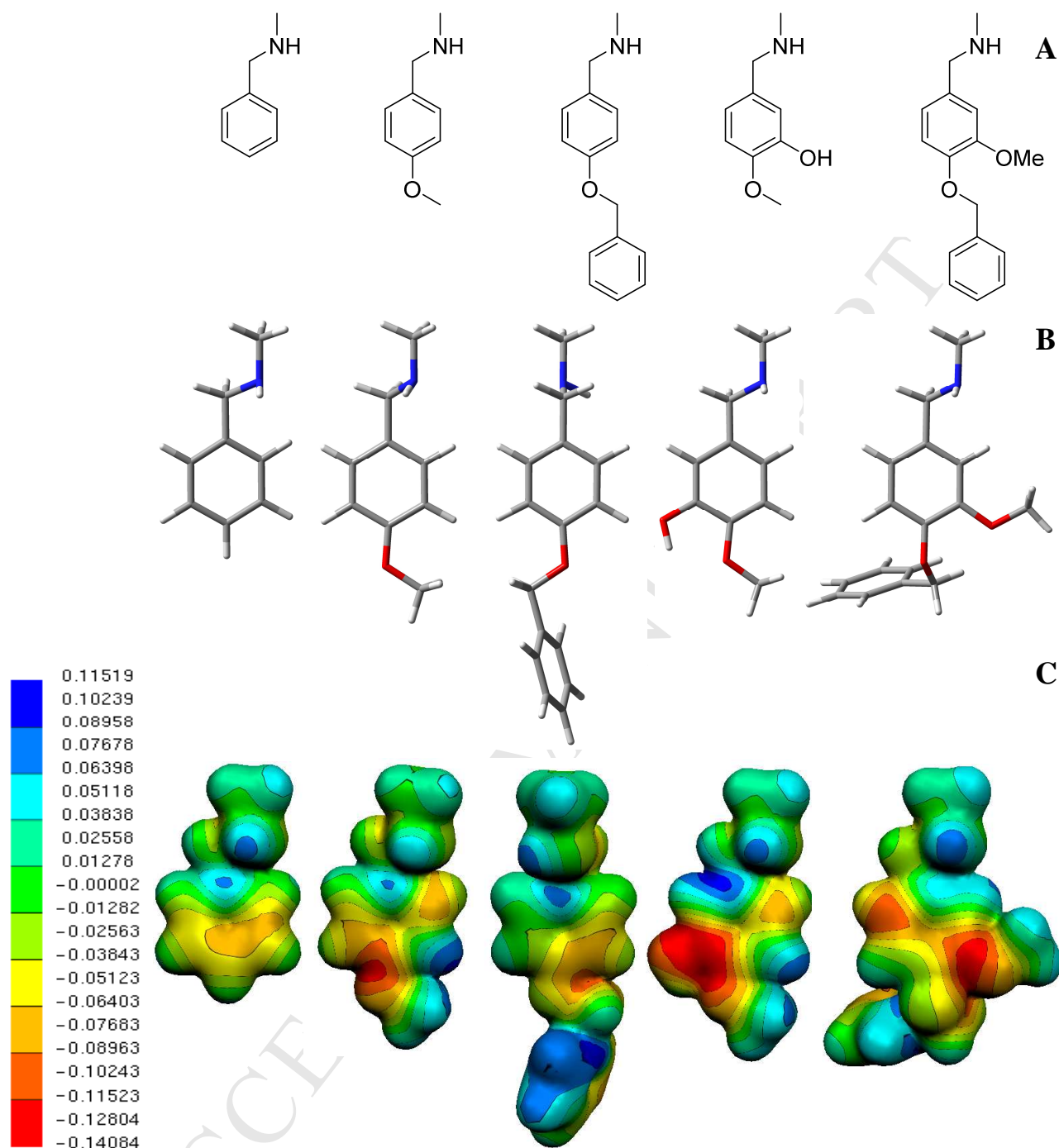


Figure 6. N-Methyl benzylamine portion of the N,N'-dibenzylamines prepared. A) Structure of the five benzylamines, B) Minimum energy conformation for each compound, C) Molecular electronic potential maps onto a van der Waals surface (isodensity 0.001 e/au³) for the N-Methyl benzylamines. The color-coded is shown at the left (in the range of 0.1408 au (deepest red) to +0.1152 au (deepest blue)).

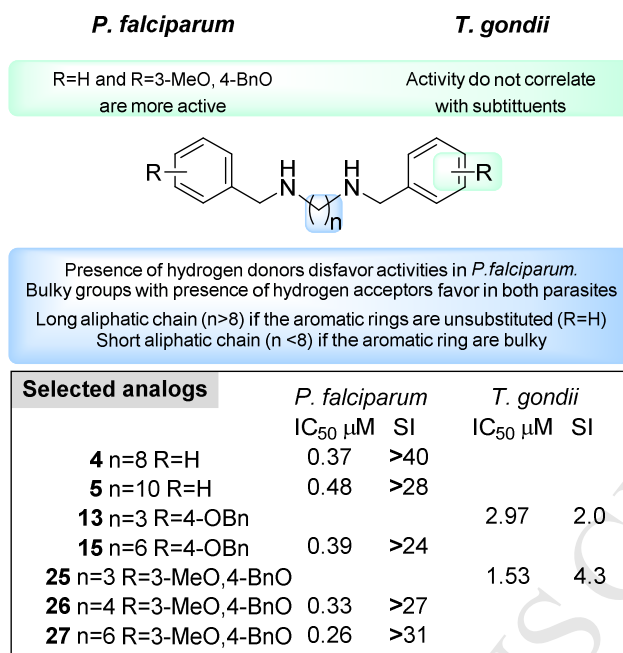


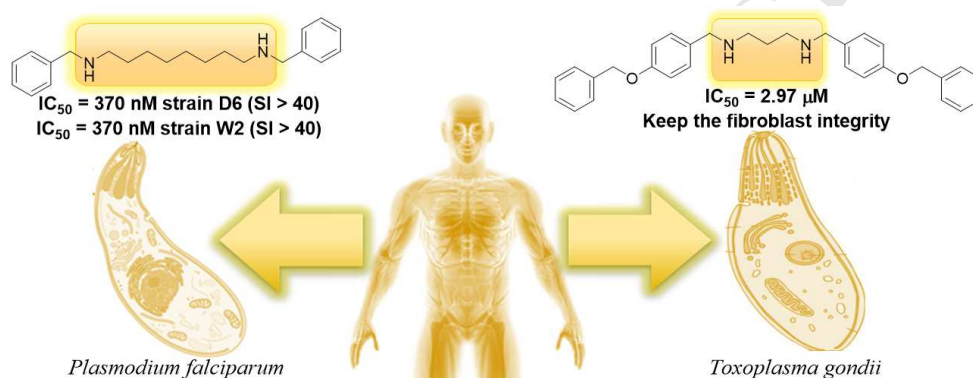
Figure 7. Structure-activity relationship of our library against apicomplexan and selected analogs.

Graphical Abstract

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A minimalistic approach to develop new anti-apicomplexa polyamines analogs.

Esteban A. Panozzo-Zénere, Exequiel O. J. Porta, Gustavo Arrizabalaga, Lucía Fagnoli, Shabana I. Khan, Babu L. Tekwani and Guillermo R. Labadie.



Highlights

- A series of N,N'-disubstituted aliphatic diamines and N,N,N',N'-tetrasubstituted aliphatic diamines were designed and synthesized as potential new chemical entities for malaria and toxoplasmosis drug development.
- All compounds were tested in *Toxoplasma gondii* and in chloroquine-sensitive and resistant-strains of *Plasmodium falciparum*.
- Most of the synthesized compounds showed potent activity in *P. falciparum*, and can be used as a starting point for the development of new antimalarial drugs. On the other hand, none of the tetrasubstituted analogues were active.
- The collection did not show the same potency on *T. gondii* and was different when compared with the malaria parasite.
- Theoretical studies were carried out to show the electronic differences between the synthesized compounds. A possible multitarget action mechanism have been proposed.